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CONTENTS

Biochemical and Nutritional studies on East Pakistan Fish. Part III.—Investigation on the Mechanism of Fish Spoilage by Study of the Influence of Visceral Contents and Boiling Treatment on the Autodehydrogenase Activity of Fish Tissues.....M. Qudrat-i-Khuda, H. N. De and N. M. Khan 79

Studies on Colchicine Induced Tetraploids in Ricinus Communis L......A. S. Islam and Aziz A. Khan 83

Development of Rapid Methods for the Estimation of the Oil Content of Single Cottonseeds. Part III.—Critical Examination of the Oil-Expression Method....S. Nurul Ahmad, Saeed A. K. Lodhi and Mazhar Mahmood Qurashi 87

The Relationship between Viscosity and Molecular Structure. Part IV.—Observation of Energy-level Structure in the Intermolecular Potential Energy of Activation for Viscous Flow of Water.....Mazhar Mahmood Qurashi and A. K. M. Ahsanullah 93

Note on a Fully Dynamical Variant of Lees' Disc Method for the Rapid Determination of the Thermal Conductivity of Poor Conductors.....Mazhar Mahmood Qurashi, A. K. M. Ahsanullah and S. H. Rizvi 96

Studies in the Relationship between Viscosity and Molecular Structure. Part V.—Preliminary Investigation of Periodicity in the Temperature Variation of the Activation Energy in Aqueous Ethyl Alcohol in the Temperature Range of 50°C. to 55°C...... A. Momin Chowdhry, Hamid Ahmad and Mazhar Mahmood Qurashi 101

Chemical Studies in Relation to the Biosynthesis of Some Natural Benzofurans and Related Compounds. Part I......B. I. Nurunnabi 108

Studies on Centaurea Behen Linn (Compositae). Part II......Nisar Ahmad and Georg Hahn 115

An Examination of Amoora Rohituka. Part I.—Constituents of the Seed.....M. Qudrat-i-Khuda and Munzural Hosain 122

Short Communications:

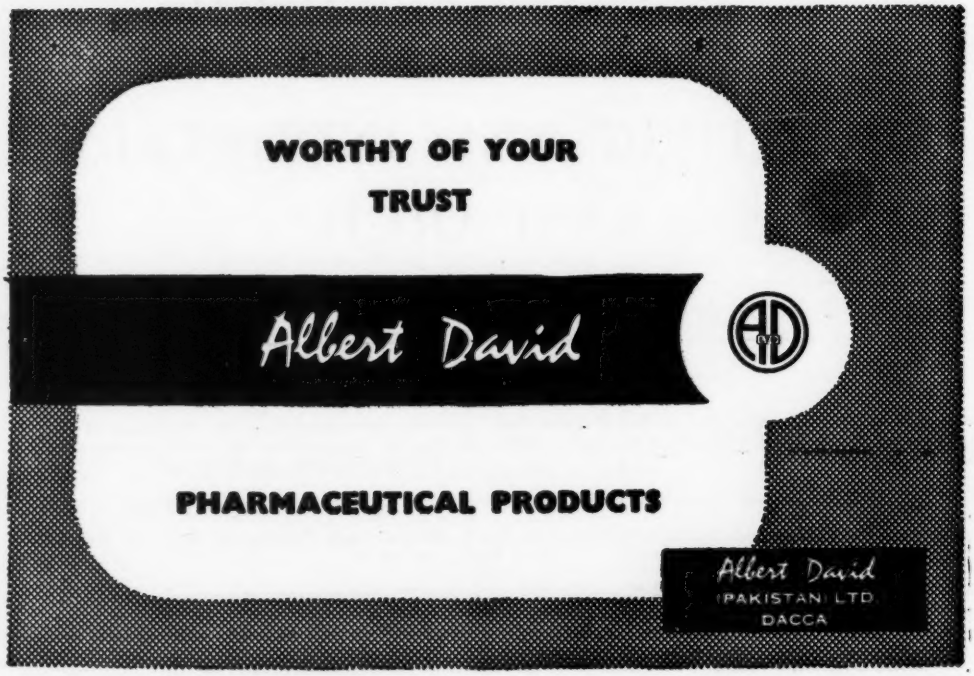
A New Method for Refining of Shark Liver Oil on Commercial Scale and Recovery of Vitamin 'A' by Partial Saponification for Production of Standard Quality as well as Higher Vitamin 'A' Potency Oil.....S. A. Ali and L. Rahman 125

Eublemma Amabilis.....S. Mahdihassan 126

Essential Fatty Nutrients in Diets and their Effects on Health, and on Liver and Heart Degeneration in Mice.....N. A. Khan 128

Notes and News..... 131

Book Notices..... 133



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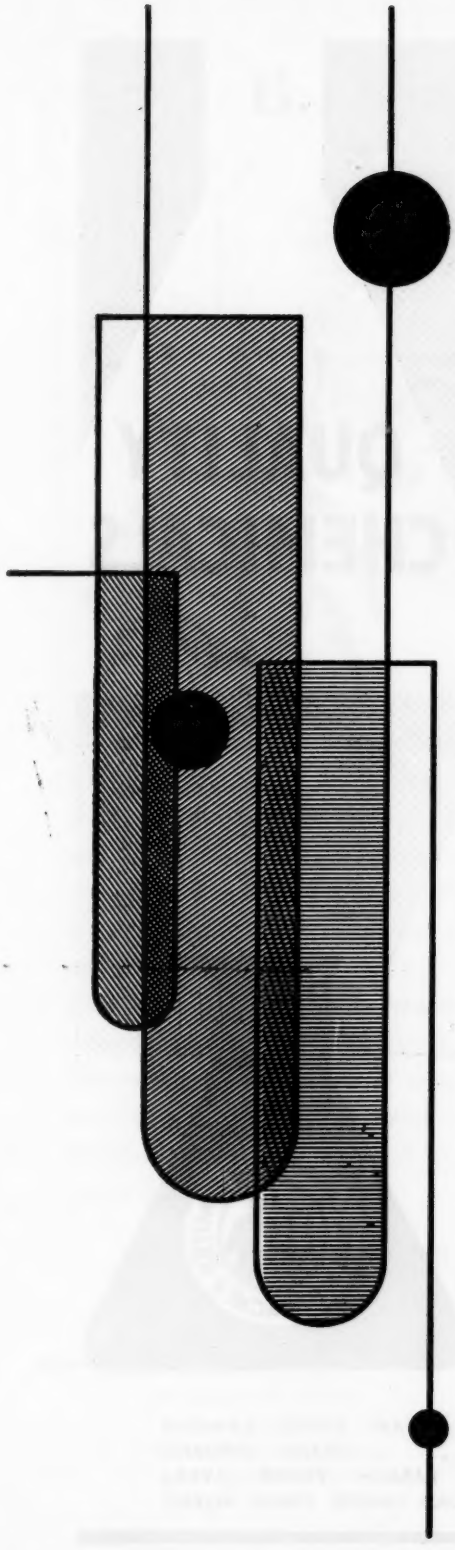

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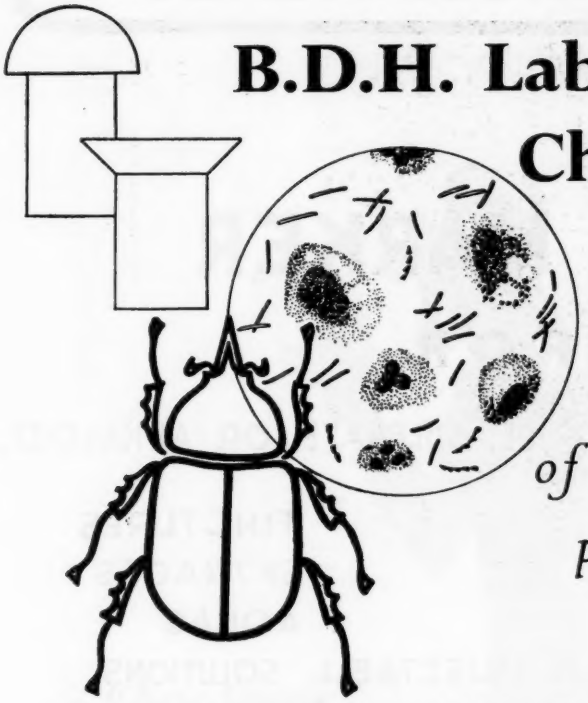

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PAKISTAN JOURNAL
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BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part III.—Investigation on the Mechanism of Fish Spoilage by Study of the Influence of Visceral Contents and Boiling Treatment on the Autodehydrogenase Activity of Fish Tissues

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(Received December 14, 1959)

It has been found that both viscera and the fillet of a fish act in a symbiotic manner, with the onset of spoilage by generation of dehydrogenases. The liver in normal condition possesses high dehydrogenase activity, indicating its rapid autolysis; and the spoilage in the fleshy portion is partly due to the influence of visceral autolysis and partly due to bacterial invasion from air.

Introduction

In the previous paper it has been shown by Qudrat-i-Khuda, De and Khan[1] that during spoilage of whole fish there is generation of the activity of total dehydrogenases in the fillet, the rate of which, however, depends on the nature of fish as well as on the total titratable acidity at the initial stage before spoilage had started. The present investigation was undertaken to see how far the presence of viscera in the intact fish influenced such generation of dehydrogenases during spoilage in the fillet and also to see whether storage after boiling may help the delay of the onset of spoilage by retarding the generation of dehydrogenases or their activities.

It has been noted that the powerful digestive enzymes in the gut even at ice temperature perforate its wall and these along with bacteria from the gut carry on the decomposition of belly wall as well as of the viscera.[2] The viscera itself has a high rate of autolysis and this combined with the above enzymes and bacteria from gut attack the belly and render it semi-fluid. Jacob[3] in discussing the above aspects has remarked that in case of uneviscerated fish autolysis may be considerable and general softening of flesh adjacent to the visceral cavity occurs. Although by gutting of fish, visceral autolysis may be completely retarded, it is still a debatable issue as to whether this process will also help in the retardation or delay in the onset of spoilage in the fleshy portion. Jacob[3] in the same review has opined that this process of gutting may contribute to a more rapid bacterial decomposition of the fillet at a later stage and this is probably because of the exposure of a large surface of the interior of the belly to the air. Dyer and Dyer[4] in their work on white fish have noted that the fillet of gutted fish might have higher content of spoilage products than similar ungutted fish. This, they attribute, is due to damage caused to intestine by such gutting process for which the spoilage products formed within the intestine diffuse into the belly cavity. Experiments with mackerol, on the contrary, did not show any difference between gutted and ungutted fish which had been iced for 6 days.[5]

From all the above reports, although it seems very possible that softening of flesh is partly due to the effect of visceral contents, yet the major decomposition of the fillet occurs due to the influence of bacteria from air or their enzymes as has been observed by a number of workers.[6,7]

The present investigation has been undertaken to have a clear picture of the above aspect

specially with regard to the individual or/and mutual influence of fillet as well as of the viscera on the spoilage mechanism in the intact fish.

Experimental

Fresh fish was collected from the market early in the morning, about 3 hours after catch. Immediately they were divided into different batches—each batch having at least three fish of equal size and all these batches were then subjected to the following series of experiments.

Experiment 1.—One batch of fish was sliced immediately after purchase and the auto-hydrogenase activity of liver as well as of the fillet was determined.

Experiment 2.—A batch of intact fish was stored under ordinary condition (80-84°F.) for 24 hours and then the liver and the fillet were analysed for dehydrogenase activity. By such storage condition the fillet became semi-fluid and emitted putrid odour after 24 hours and hence storage for further period was not possible.

Experiment 3.—At the beginning of experiment, the fish was eviscerated and both the liver and the fillet were stored separately in the same manner as before, after which the enzyme activity was recorded at different storage periods. Since under such storage condition the fish could be stored for 72 hours, the activity up to that stage of storage could be recorded easily.

Experiment 4.—One batch of fresh intact fish was boiled and stored under laboratory condition as before and thereafter the enzyme activities of both liver and flesh were determined.

Experiment 5.—The intact fish after boiling was eviscerated and both boiled liver and fillet were stored separately and thereafter their dehydrogenase activities were recorded at each stage of storage.

Experiment 6.—In this batch the viscera was removed and the eviscerated fish was then boiled. Unboiled viscera and boiled fillet were then stored separately and their activities determined after storage.

Technique of Evisceration.—Evisceration of the whole intact fish was done by making a small horizontal opening by sharp knife at the junction of the head and the belly. This process of evisceration is followed in this part of the country to minimize the chances of exposure of the fleshy portion surrounding the belly to bacterial invasion. In other countries evisceration is done by completely opening the belly, thus exposing the entire belly wall to bacterial attack for which the real autolytic effect of viscera on the spoilage of fillet cannot be fully judged. The process of evisceration adopted in the present investigation is expected to yield better information on the above aspect.

The dehydrogenase activity of 1 g. tissue sample was determined according to the technique detailed in our previous paper.[1] It may be noted here that for analysis of the fillet, the portion below the back bone and above the belly was collected from each sample. All the analyses were done in replicate and the difference in such replicate values was not much significant under the condition in which the above experiments were conducted.

Three types of fishes like Rohu Naola (below 10 inches) and small size fishes like Puti and Chapila were selected for the work. Since in all the three cases the effect of the above treatments was almost the same, specimen data for Rohu Naola only are given here.

Results

The results of the experiments presented in Table 1 show that in Experiment 1, the flesh at the initial stage just after purchase did not show any appreciable autodehydrogenase activity, whereas the liver, immediately after removal showed a certain amount of activity.

After 24 hours storage of intact fish (Experiment 2) the autodehydrogenase activity of liver tissue separated from the fillet after storage increased by nearly 3 times from the original value and that of the fillet approached to a considerable amount from almost negligible value.

The results of Experiment 3, in which eviscerated fillet and the separated liver were stored individually, indicate that the fillets of eviscerated fish after 24 hours storage also elaborated sufficient dehydrogenase activity but the rate in such case was much lower than that of the fillets of uneviscerated fish of Experiment 2. The extent of apparent inhibition of activity in this case was such that rate of activity after 48 hours storage did not even approach the limit as was elaborated in case of uneviscerated fillet after 24 hours storage. In contrast to the fillets, the separated liver of eviscerated fish on storage, however, showed rather increase in the values as compared to that of uneviscerated fish so much so that after 48 hours storage the activity almost approached to unity.

Study of the effect of pre-storage boiling treatment of intact fish on the generation of dehydro-

genase activity has shown (Experiment 4) that the inherent high activity of the enzyme in liver of fresh fish as was noted in Experiment 1 is completely destroyed during the process of boiling before any storage was started. When uneviscerated boiled fish was stored for 24 hours the activities of fillet as well as of liver, separated from each other after the above storage period, showed lesser values than those of unboiled and uneviscerated fish stored for similar period as in Experiment 2. The activities of both fillet and the liver increased with progress of storage period but it may be noted that the ultimately increased values even after 72 hours storage in this case did not reach the same limit as was noted in case of 24 hours storage of unboiled and uneviscerated fish of Experiment 2. When the fish after boiling was eviscerated (Experiment 5) and both liver and the fillet were stored separately, the dehydrogenase activity of both these tissues showed much lower values as compared to those of boiled and uneviscerated fish as recorded in Experiment 4. When only the fillet was boiled after evisceration and both the boiled fillet and the unboiled liver were stored separately, the fillet in this case also showed lower dehydrogenase activity as compared to that of uneviscerated intact boiled fish. Liver, not being boiled in this case, behaved in a similar way as that of separated liver of eviscerated unboiled fish (Experiment 3) stored under identical conditions.

TABLE 1.—THE EFFECT OF EVISCERATION AND BOILING TREATMENT ON THE DEHYDROGENASE ACTIVITY IN FISH TISSUES (ROHU NAOLA) DURING STORAGE.

Experiment No.	Nature of treatment	Duration of storage in hours	Nature of tissue studied	Time of decolorisation of M.B. per gm. tissue in minutes (T)	Activity $\frac{1}{T}$
Expt. 1	immediately after purchase	0	Flesh	More than 12 hrs.	Negligible
			Liver	25	0.04
Expt. 2	Stored as intact fish	24	Flesh	7	0.142
			Liver	8	0.125
Expt. 3	Stored after evisceration	24	Flesh	23	0.043
			Liver	6	0.167
		48	Flesh	18	0.055
			Liver	Instantaneous	1.00
		72	Flesh	10	0.10
			Liver	Totally decomposed	
Expt. 4	Stored as intact fish after boiling	0	Flesh	Decolorised after 24 hrs.	Negligible
			Liver	,,	,,
		24	Flesh	23	0.043
			Liver	10	0.10
		48	Flesh	16	0.062
			Liver	8	0.125
		72	Flesh	7	0.142
			Liver	5	0.20
Expt. 5	Fish boiled, eviscerated and stored	24	Flesh	37	0.027
			Liver	29	0.034
		48	Flesh	28	0.036
			Liver	22	0.045
Expt. 6	Eviscerated fish, boiled and stored	24	Flesh	37	0.27
			Liver	5	0.02
		48	Flesh	18	0.055
			Liver	Instantaneous	1.00

Discussion

The results of Experiment 1, clearly indicate that the fillets of the Rohu Naola although do not possess any appreciable amount of dehydrogenase activity at the initial stage just after purchase from the market, the liver tissue, on the contrary, yields evidence of the presence of high level of these enzymes. This is indicative of the survival stage of the liver tissue even under apparently dead condition of the intact fish and this is perhaps the cause of autolysis of the visceral contents. This process of autolysis involves the participation of a large number of dehydrogenases and other enzymes for the performance of glycolysis, Kreb's cycle oxidation and other processes for the breakdown of different constituents.

The results of Experiments 2 and 3, in which it was observed that fillets of unevisecrated fish on storage at the room temperature of 80°-84°F. elaborated comparatively more activity than that of eviscerated fish stored under similar conditions, suggest that the generation of dehydrogenases in fillet of intact fish is not only the result of decomposition by bacteria invading through the skin and other routes but may also partly be due to the effect of liver from which its autolytic enzymes along with the food bacteria of the gut or their enzymes reaching the liver after penetration

through the intestinal wall, might have diffused into the fillet and had accelerated the spoilage process there.

Whereas the results of Experiments 2 and 3 indicate the influence of visceral contents (liver) on the spoilage of fillets, those of Experiments 4, 5 and 6, on the contrary, submit a new picture regarding the influence of fillet on the decomposition of visceral contents. The observation as to the complete destruction of dehydrogenase activity of liver tissue after boiling treatment, the regeneration of the activity of both fillets and liver of boiled uneviscerated fish on storage and comparatively lesser generation in both the above tissues in case of boiled but eviscerated fish, suggest that the enzymes of the liver of fresh fish even after complete destruction or inhibition in their activities due to boiling treatment are regenerated when left in contact with the fillet in intact fish and this is probably due to the influence of fillet. While the liver remains within the belly under sterile condition after boiling treatment, the fillet on the contrary even after sterilisation by boiling remains exposed to the air and thus the bacteria from air get access into the fillets through skin and other routes and initiate decomposition therein. The enzymes of these bacteria of fillets perhaps diffuse into the liver and other visceral contents and initiate the spoilage in this tissue also.

Summarising the results of the above experiments it may be postulated that both the fillet as well as the viscera (liver) act in a symbiotic manner in the causation of spoilage of whole fish when stored under ordinary atmospheric condition in the laboratory.

This symbiosis with respect to the mechanism of fish spoilage will depend on the rate of autolysis of the liver and other visceral contents coupled with the presence of intestinal bacteria on the one hand, and on the extent of bacterial invasion in the fillets on the other.

Further work is in progress for investigating the influence of evisceration process adopted in other countries on the spoilage of fish so that the right process of evisceration to cause less spoilage in the fillet may be evaluated.

Acknowledgement.—The authors express their thanks to Dr. S. Siddiqui for his interest in this work.

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STUDIES ON COLCHICINE INDUCED TETRAPLOIDS IN RICINUS COMMUNIS L.

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(Received November 18, 1959)

On the assumption that duplication of chromosomes would increase the oil content of the seeds of *Ricinus Communis L.* tetraploids were otbained by colchicine treatment. It was however found that in spite of the fact that the size of the seed had increased the process is uneconomical due to late flowering, the failure of tetraploid to form fruits without hand pollination and very small number of seeds produced.

Introduction

As duplication of chromosomes is reported to bring about an increase in the oil content in some oilseed crops,[1] it was thought that similar duplication of chromosomes in *Ricinus communis* L. would increase its oil content. Cross pollinated plants usually respond more favourably to the effect of chromosome doubling and from this point of view as well *R. communis* was found to be a suitable material.

The present paper deals with the preliminary results obtained in the production of tetraploid *R. communis* by means of colchicine treatment.

Methods and Materials

Seeds were collected locally from two varieties of *R. communis*, namely, green and red. Chromosomes were studied from pollen mother cells in aceto-carmine preparations.

Colchicine Treatment.—The freshly germinating seedlings, from which the endosperm was removed, were dipped in a 0.2 per cent aqueous colchicine solution for a duration of 40 and 48 hours. The temperature ranged between 70 and 85°F. at the time of treatment. The results are given in Table 1.

These results show that no seedlings survived the treatment lasting for 48 hours. Of the 50 seedlings treated for 40 hours, 30 survived and of these 3 proved to be tetraploids from stomatal and pollen grains studies.

Table 1.—Results of Treatment with a 0.2 per cent Colchicine Solution.

Details	Red		green		Total	
	40 hrs	48 hrs	40 hrs	48 hrs	40 hrs	48 hrs
1. Number of seedlings treated	30	15	20	15	50	30
2. Number of survivals	18	—	12	—	30	—
3. Number of tetraploids obtained	2	—	1	—	3	—

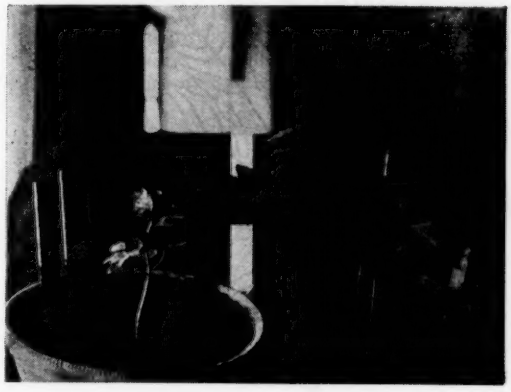
Morphology of the Treated Plants.—

1. Cotyledons: All the treated seedlings showed greatly distorted and reduced cotyledons (Fig. 1).

2. Height: All the treated plants were shorter than their diploid counterparts. Table 2 shows

1A 1B 1C

Fig. 1.—Cotyledons: A. Control; B. and C. Treated. ×¾.



A B

Fig. 2.—Height: A, Treated; and B, Control, × 6¾

Table 2.—Comparison of Characteristics in Treated and Control Plants.

Characteristics	Red		Green	
	Control	Treated	Control	Treated
1. Height in inches..	29.28± 4.15	27.56± 4.75	26.00± 3.27	23.12± 2.49
2. Leaves				
(a) B/L ratio	1.31	1.34	1.29	1.32
(b) B/L ratio of guard cells ..	0.58	0.63	0.61	0.67
(c) Number of stomata per unit area	10.44± 1.00	7.68± 0.83	11.30± 1.04	8.78± 1.78
3. Diameter of pollen grains in μ ..	27.45± 2.30	35.12± 4.52	27.07± 1.59	35.82± 3.11
4. Sterility percentage in pollen grains	3.89	12.50	4.01	10.01

their comparative heights (Fig. 2).

3. Leaves: The leaves of the treated plants showed the usual induced tetraploid characteristics, i.e., higher B/L ratio, and thicker and darker green leaves. The venation was more pronounced in the treated plants. The leaf size increased both in length and breadth but the increase was greater in breadth, resulting in higher B/L index (Table 2). The laciniation of leaves was shorter and the width of the leaf lobes was greater in the treated than in the control plants (Fig. 3A and 3B).

4. Stomata: As is usual with the induced polyploids, the leaf guard cells in the treated plants showed an increase in the dimensions of the guard cells with consequent reduction in the number of stomata per unit area (=0.0008 sq. inch) (Table 2). Figures 4A and 4B show a comparative size of stomata in the diploid and tetraploid plants.

5. Inflorescence: The peduncle of the inflore-

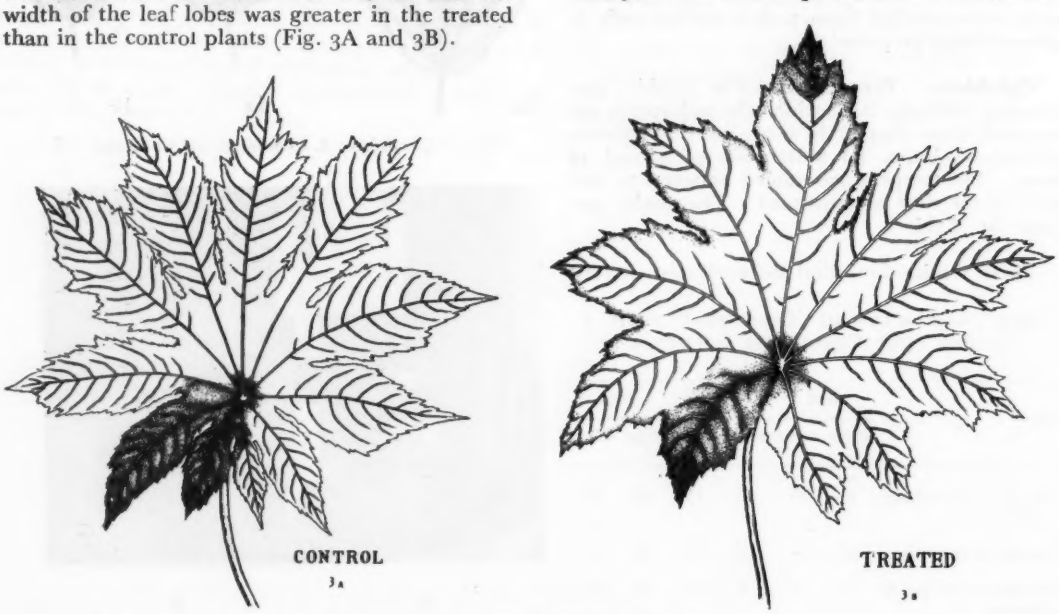
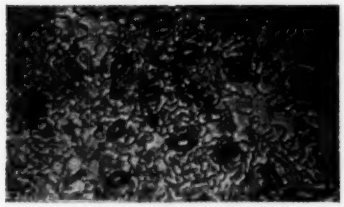
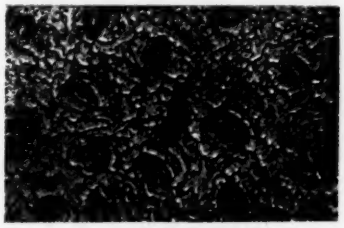


Fig. 3.—Leaves: A, Control; and B, Treated.



(A)

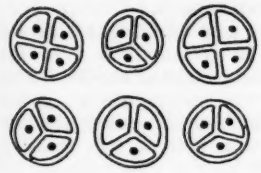


(B)

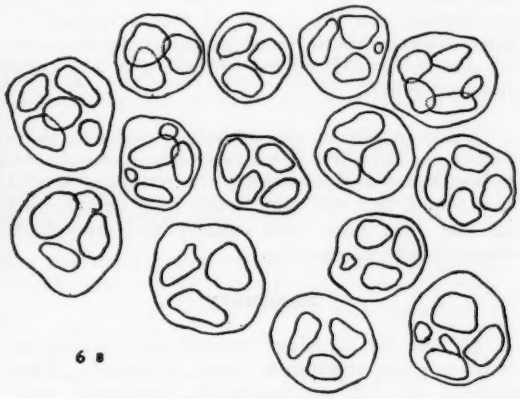
Fig. 4.—Stomata: A, Treated ; and B, Control, × 280.

5 A 5 B

Fig. 5.—Seeds: A, Control; and B, Treated × ¾.



(A)



6 B

(B)

Fig. 6.—Tetrahedral and isobilateral tetrads in: A, Control; and B, Treated, × 500.

scence was shorter and with a smaller number of flowers as compared to the control.

6. Pollen grains: Ten to twelve per cent unstainable pollen grains were noticed in the treated plants (Table 2). They also showed large variations in their diameter. The percentage of stainability varied a great deal from plant to plant and even among different flowers of the same plant.

7. Fruit: In spite of a large number of pollen grains having good stainability in the tetraploids, there was seldom any fruit formation without hand pollination. The number of fruits in the tetraploids ranged from 4 to 6 as compared to 16 to 40 fruits per peduncle in the control. The fruits in the tetraploids were, however, slightly larger.

8. Seeds: Only one seed was found to develop per fruit in the tetraploid plants so far collected as against 3 in the control. It thus appears that of the three ovules in an ovary only one became fertilized in the tetraploids. The seeds in the treated plants were almost double the size of those in the control (Figs. 5A and 5B). The only 6 seeds obtained from the tetraploids weighed 2.1 g. as against 1.66 g. being the weight of the largest 6 seeds from the control.

9. Flowering Time: Flowering time of the treated plants was delayed by about 2 months. In blooming later they behaved like the majority of induced polyploids.[2]

Meiosis.—The gametic chromosome number was confirmed to be 10 as reported by Hagerup.[3] One interesting point observed was the presence of two types of tetrads, namely, tetrahedral and isobilateral in the ratio of 2:1 (Figs. 6A and 6B). In the treated plants the ratio was almost changed to 1:1 (Table 3). This change of ratio is probably due to the disturbance in meiosis brought about by chromosome doubling. In tetraploid plants different types of sporads were found.

TABLE 3.—NUMBERS OF TETRAHEDRAL AND ISOBILATERAL TETRADS IN DIPLOID AND TETRAPLOID PLANTS.

Types of tetrads	Diploid	Tetraploid
Tetrahedral	215	87
Isobilateral	107	83
Total	322	170

Discussion

The only previous work on experimentally produced tetraploids was by Sidorov and Sokolov.[4]

Out of 248 plants treated with colchicine they obtained only 3 tetraploid plants. The proportion of tetraploids in our case was higher, as we obtained 3 tetraploids in a population of 80 plants. Except in size and weight of the seeds the tetraploids proved to be very uneconomical, as all of them flowered late and had a smaller number of flowers per inflorescence with a still smaller number of fruits because of the development of only one seed per fruit. What was more disappointing was the failure of the tetraploids to form fruits without hand pollination; this may be due to the differential behaviour of the pollinating agents.

Since in almost all artificially induced polyploids the fertility is considerably increased by hybridization and selection at the tetraploid level,[5] it is hoped that the same result may be obtained in this case also, thus making the tetraploids economically productive.

Summary

Of the 80 seedlings treated with colchicine only 3 pure tetraploids were obtained. They showed all the characteristics associated with induced polyploids, namely, thicker and darker green leaves with pronounced venation, larger and fewer guard cells, larger pollen diameter with higher sterility, larger but fewer fruits and larger and fewer seeds per fruit.

All the 3 tetraploids, however, proved to be uneconomical due to shy bearing. Methods of improvement are discussed.

Acknowledgement.—The authors acknowledge the services of Mr. E. H. Naqvi for the drawings and of Mr. Manzoorul Imam for going through the manuscript.

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DEVELOPMENT OF RAPID METHODS FOR THE ESTIMATION OF THE OIL CONTENT OF SINGLE COTTONSEEDS

Part III.—Critical Examination of the Oil-expression Method

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The method utilizing the expression of oil between several discs of filter paper has been exhaustively tested, and a detailed assessment of the conditions governing its accurate utilization is made. The law connecting the area of the oil spot with crushing load is obtained and a technique of correcting for the varying water content of the kernel is worked out. The influence of the wetness of the filter discs has been separately determined, and measurements at an optimum relative humidity of about 80% are recommended.

While the diameter and grade (i.e. coarseness or fineness) of the filter paper are shown to have a very small effect, of the order of ± 3%, on the results, the size of the oil spot is found to be inversely proportional to weight per unit area of the discs, as was anticipated from theory. This makes it possible to directly correlate measurements made with different types of filter papers. The most favourable conditions for reproducibility and accuracy are noted, and a constant is obtained for direct conversion of area of oil spot into milligrams of oil present in the kernel.

1. Introduction

The rapid estimation of oil content on small samples of cottonseeds is a matter of considerable importance for the selection of suitable batches of seeds for propagation in experiments on the breeding of high-oil-content varieties. The smallest possible sample appears to be a single cottonseed, and in an earlier communication,[1] experimental results were presented to show the feasibility of determining the oil content of a single cottonseed by expression of the oil between several discs of filter paper.

The kernel from the dehulled cottonseed is placed between several discs of filter paper, an equal number on either side, and the sandwich is then placed between the plattens of an ordinary 10-ton laboratory hydraulic press. A suitable crushing load is applied, and on removal from the press, the sandwich presents the appearance shown diagrammatically in Fig. 1 (top). The dark patch in the centre is the crushed non-oily portion of the kernel and surrounding this is the slightly elliptical spot made by the expelled oil, the edges of which spot are very sharp, allowing accurate measurement of its diameters to 0.1 mm. with a low-power microscope or by estimation with a millimetre scale. The mean of two mutually perpendicular diameters leads to an exact value of the total area of the spot. Errors of measurement are further reduced by repeating the measurements on several of the filter papers forming the sandwich. With six filter dics, the area of the oil spot is of the order of 200 sq. mm. for the average cottonseed.

This method has now been tested exhaustively and in the present paper, a detailed assessment of the conditions governing its accurate utilization is made, and a value is obtained for the conversion factor connecting the oil content with the effective area of the oil spot.

2. Law connecting the Crushing Load with the Area of the Oil Spot

In the experiments described in the previous communication,[1] cottonseeds of the L. S. S. variety were mostly used, and it was found (Fig. 1 (bottom)) that, in the range of 5 to 10 tons crushing load, a maximum sensitivity, defined as area of

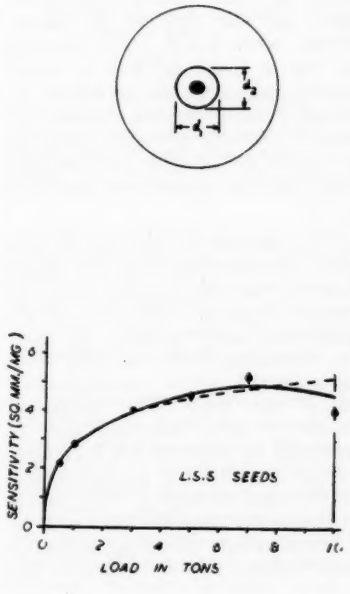

Fig. 1. (Top) Diagramatic sketch of the filter discs with crushed kernel and oil spot. (Bottom) Previously reported plot of sensitivity (i.e. area of oil spot per mg. of kernel) against load, obtained with L. S. S. Cottonseeds.

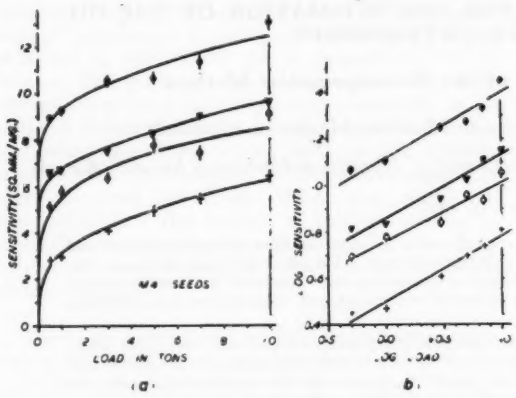

Fig. 2.—Plots of measurements with several lots of M4 variety of cottonseeds for determining the law for the sensitivity-load curve: (a) sensitivity against load and (b) logarithm of the sensitivity against logarithm of the load, the latter yielding straight lines in every case.

oil spot per mg. of kernel, was obtained. Since there are indications of unusually large variations in the percentage oil content of seeds of the L. S. S. variety,[2] it was considered desirable first to check the previous measurements by working with another variety, namely M 4. Typical sets of readings obtained with several lots of (24) M 4 cottonseeds under varying conditions (at different times of the year) are shown in Fig. 2(a), and they all indicate a slow monotonic increase in area of oil spot with increasing load, and this feature has been confirmed in all the other varieties examined. The precise reason for the shallow maximum previously observed with L.S.S. seeds has not been ascertained, but it could be due to some unduly large, positive and negative variations in the readings at 7 and 10 tons, respectively, because a curve in reasonable concordance with those for M 4 seeds is obtained by giving these last readings less weights as shown by the broken line in Fig. 1 (bottom).

At all events, the monotonic increase in area with crushing load is reasonable if we remember that a fixed volume of the oil expelled from the kernel has been absorbed on the filter discs, which are now being uniformly compressed by the load. The reduction in thickness of the oil film must therefore be compensated by a corresponding increase in area of the spot. The analytical form of the relation between load and area of spot can be determined by plotting the logarithm of the sensitivity i.e. area/mg. of kernel against the logarithm of the load, as in Fig. 2(b), where successive sets of points are displaced upwards by 0.2 unit. The sets of points give approximately linear and parallel plots, whose equations can all be represented by

$$\log S = A + B \log (\text{Load}/10), \qquad (1)$$

where B lies between 0.25 and 0.29 and A (i.e. $\log S_{10\ \text{tons}}$) varies from 0.73 to 0.81, the mean values being 0.27 ± 0.01 and 0.78 ± 0.03, respectively. Thus, from equation (1) we can get

$$S = 10^{0.78} \times (\text{Load}/10)^{0.27} = 6.0 \times (\text{Load}/10)^{0.27} \qquad (2)$$

as the mean equation of the sensitivity curves of Fig. 2(a).

The variation in the value of A for the individual graphs of Fig. 2(b) is considerably greater than might reasonably be expected from the estimated standard errors of the experimental points, and further examination of the data showed that the lowest value (0.73) corresponds to the data (triangles) obtained under a prevailing relative humidity of about 45%, while the highest value (0.81) is obtained from the experiment (hollow circles) carried out at a time of the year when the relative humidity was nearly 85%. This correlation suggested a more detailed study of the influence of atmospheric humidity on the area of the oil spot obtained at different loads. This influence can be two-fold, viz.

(a) increase in area of oil spot due to increased water content of the kernel,
(b) variation produced by a change in the compressibility and porosity of the filter paper on exposure to high humidities,

and those two effects are investigated separately in the following.

3. Influence of the Water Content of the Kernel and Postulation of "Effective Area of Oil Spot"

For this investigation, an auxiliary experiment was first conducted to determine the relationship between the relative humidity and the water-content of the kernels used (M 4 variety of cottonseed). Several humidifiers, each giving a specified relative humidity, were made by placing saturated solutions of various salts (in place of the usual desiccant) in a series of desiccators. Two sets of three kernels of M 4 cottonseeds were then run through one complete hydration $\rightleftharpoons$ dehydration cycle, starting with decreasing humidity for one set and increasing humidity for the other, and the weight of each kernel was recorded at each of the five humidities available. The mean results obtained as weight % water are plotted in Fig. 3, together with the corresponding data for the cottonseed cake (broken line) from seeds of the M 4 variety, the vertical lines through the plotted

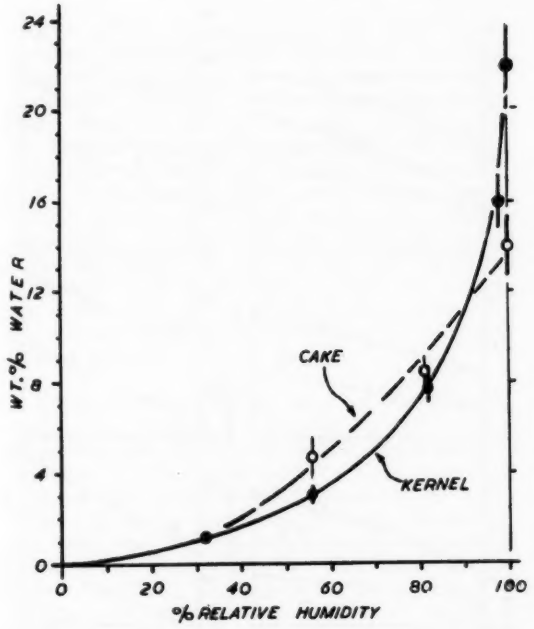

Fig. 3.—Graphs showing the measured variation of water content of cottonseed kernel (solid circles, full line) and cake (hollow circles, broken line), plotted against percent relative humidity at an ambient temperature of 26°C. The steep rise in the right half of the graph is to be noted.

points indicating the experimentally estimated standard errors. It is seen that up to a relative humidity of 70%, the water-content of the kernels increases rather slowly, and thereafter rises rapidly to over 20% at 100% humidity. On the basis of this graph, a suitable distribution of humidities was selected for the main experiment, and values of 60% and 90% were fixed upon between the extreme values of 0% and 100%, and four desiccators were arranged to give nearly these four values of humidity. The actual values of the humidity obtained were 0%, 56 ± 1%, 89 ± 1% and 100%. In addition, some readings were taken using the atmospheric humidity of 85 ± 2%.

Five lots of 24 kernels of the M 4 variety of cottonseeds were placed in these five humidities, and the kernels were removed one by one after a period of 30 hours, which sufficed for the attainment of equilibrium. Each kernel was weighed and then placed in a sandwich of six filter papers (Greens 401, dia. = 11 cm.) and crushed at the appropriate load, after which the mean diameters of the oil spot as well as the crushed kernel were measured to 0.1 mm. The room temperature at this time was 26° to 28°C. and the atmospheric

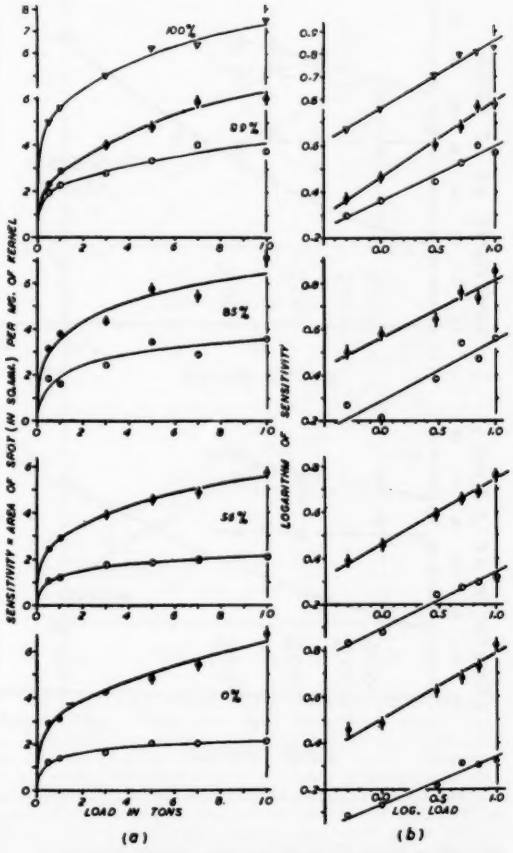

Fig. 4.—(a) Normal plots and (b) logarithmic plots for sensitivity against load, obtained with M4 cottonseed kernels maintained at several different relative humidities (noted as % in the figure), the filter papers being kept at the ambient humidity of 85% ± 2%. Solid circles for area of oil spot, hollow circles for area of crushed kernel (triangles at 100% relative humidity).

relative humidity was 85 ± 2%. The results of these measurements are plotted on Fig. 4(a) in terms of the areas of oil spot and of crushed kernel per mg. of kernel, the abcissae being the crushing loads used. Fig. 4(b) shows the corresponding logarithmic plots.

All the curves have essentially the same shape, and can be fitted by equations of the type log S=A+B log (Load/10). We may therefore use the value of S at a suitably selected load, say 7 tons, for a detailed analysis of the effect of humidity. In Fig. 5 (top half) are shown the values of S_7 for the oil spot (hollow circles) and of the corresponding quantity for the area of crushed kernel (S_7, crosses) against relative humidity and water content of

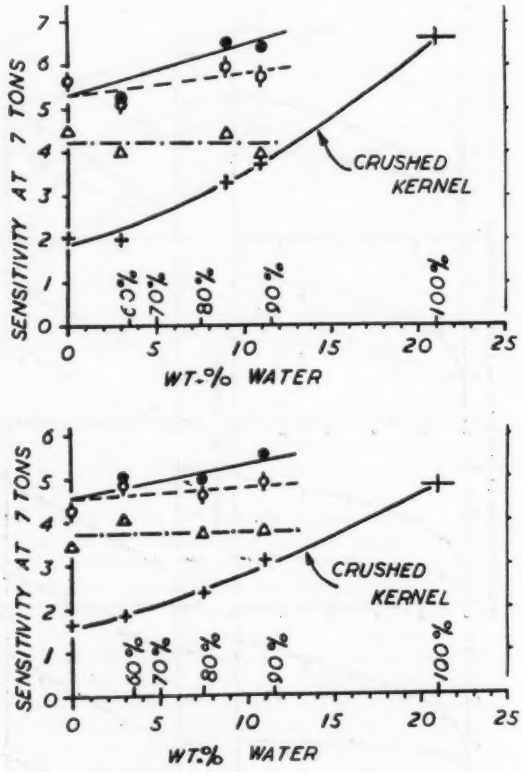

Fig. 5.—Plots against wt.% water (in kernel) for the sensitivity at 7 tons: (i) hollow circles, on the basis of wet weight of kernels (ii) solid circles, on the basis of dry weight of kernels (iii) crosses, for the area of crushed kernels only (iv) hollow triangles, for the "Effective" area of oil spot, also on the basis of dry weight.

The upper half of the figure gives the results obtained from Fig. 4 with the filter paper at 85% relative humidity, while the lower half gives those based on Fig. 6 with the paper at 40% relative humidity.

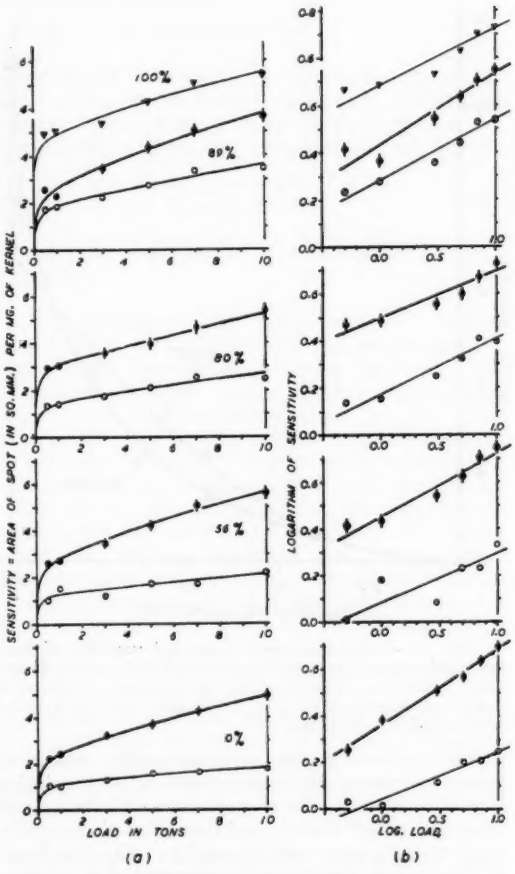

Fig. 6.—Plots as for Fig. 4, but with filter papers maintained at 40± 2% relative humidity.

the kernel. Whereas the five points for area of crushed kernel show a marked progressive increase with increasing water content, those for the area of the oil spot show a relatively smaller percentage change, at least from 0% to 89% relative humidity i.e. 0% to 11% water content. (With the fully saturated kernels, the oil spot could not be observed because of the large and uneven spread of the soft kernel.) This near constancy is however fortuitous, because the weights of the kernels include the varying weights of water absorbed under the several degrees of humidity, and when the areas of oil spot/mg. of kernel are reduced to the basis of a standard state, e.g. complete dryness (or 10% moisture content), they are found to increase substantially with water content of the kernel (Fig. 5 (top half), solid circles). This increase is, however, about 0.6 of the corresponding increase in the area of the crushed kernel.

It follows that if we define an "Effective Area of the Oil Spot" by the relation

$$A_{eff} = (A_{total} - \alpha \times A_{kernel}) \qquad (3)$$

and calculate the effective sensitivity on the basis of A_{eff} instead of A_{total}, then the effective sensitivity will be nearly independent of the water content of the kernel if $\alpha = 0.6$. The corresponding effective sensitivities are plotted as triangles in Fig. 5 (top half) and are seen to yield a horizontal graph within the limits of the experimental errors. The above result can be interpreted physically by assuming that only about half of the water in the kernel is expelled on to the filter paper, while the rest remains bound in the crushed kernel.

4. Influence of Wetness of Filter Paper

The effect of conditioning the filter paper discs at different realtive humidities has been examined by repeating the whole series of measurement under dry winter conditions when the atmospheric humidity lay in the range 40% ± 2%. The experimental curves for sensitivity as a function of load are shown in Fig. 6, while the lower half of Fig. 5 shows the corresponding plots of S_7 and S'_7 for the area of oil spot and crushed kernel, respectively, together with the "effective" sensitivities calculated with $\alpha = 0.5$ to yield a horizontal graph (hollow triangles). Thus, it is seen that a value of $\alpha = 0.55 \pm 0.05$ is adequate to correct for the variation in water content of the cottonseed kernels.

However, the absolute values of the effective sensitivities obtained are different for the two sets of experiments, being 4.15 at the higher relative humidity of 85% (upper half of Fig. 5) and only 3.72 at the lower relative humidity of 40% (lower half of Fig. 5). The difference, amounting to a little over 10%, represents the effect produced by the water absorbed in the filter paper at the higher humidity, and points to the necessity of working with filter papers maintained at a standard relative humidity if a high degree of reliability is aimed at. Since the effect will be proportional to the weight of water absorbed by the paper, rather rigid humidity control (± 2%) will be required if very high humidities (~ 90%) are employed, cf. the graph of Fig. 3. However, it seems practical to work at 80% relative humidity corresponding to the accepted standard of 10% water content for measurements on cottonseeds.

5. Effect of Diameter, Weight and Grade of Filter Paper

There remain for consideration the effects of the diameter, weight and grade of filter paper employed in this method of oil estimation. From a given packet of 11 cm. diameter filter papers, several sets of discs 5.5 cm. dia. and 3.5 cm. dia. were cut out, and measurements of the oil spot at various loads were made with these small filter discs, following the usual procedure and using a fixed relative humidity of 60%. The resulting graphs for log sensitivity against log Load are shown in the lower half of Fig. 7, and it is at once apparent that the sensitivity increases as the discs are made smaller. However, the influence of size of disc is small in the range of 11 cm. to 5.5 cm. diameter, amounting to less than 0.02 in log S_7 i.e. less than 5%. The curve for the 3.5 cm. dia. disc is uniformly higher than the others by about 0.07, corresponding to a factor of about

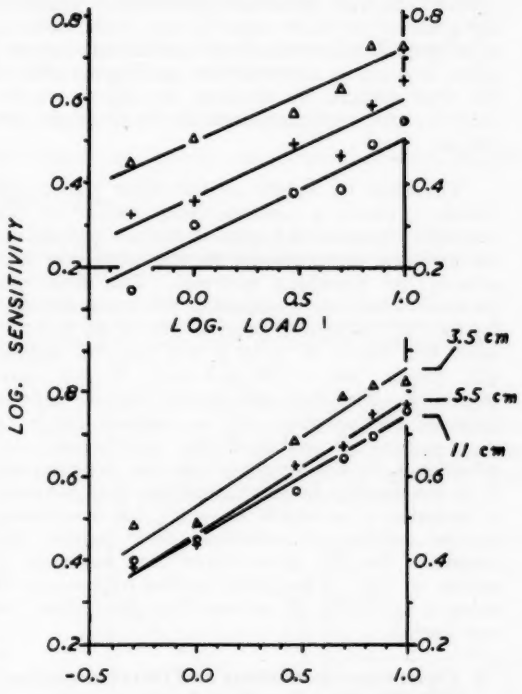

Fig. 7. (Lower half) Logarithmic sensitivity graphs obtained with three different sizes of filter discs, 11 cm., 5.5 cm., and 3.5 cm. dia. (Top half) Logarithmic "effective" sensitivity graphs for three different grades of filter papers hollow circles, Whatman No. 41; crosses, Whatman No. 1; triangles, Whatman No. 42. Successive graphs have been shifted upwards by 0.1 unit for clarity.

1.2. Thus, discs of diameter from 5 cm. to 11 cm. will be quite suitable for obtaining accurately reproducible results.

Similarly, the effect of changing the grade i.e. the texture of the filter paper was examined by obtaining logarithmic "effective" sensitivity curves (using effective area of oil spot) under constant conditions (R. H. = 81%), but using three different grades of 11 cm. diameter paper, viz. (1) Coarse (Whatman 41, wt. = 796 ± 9 mg.), (2) Medium (Whatman 1, wt. = 777 ± 8 mg.), and (3) Fine (Whatman 42, wt. = 821 ± 6 mg.). These graphs are shown in the upper half of Fig. 7, the successive plots being shifted upwards by 0.1 unit to prevent confusion due to overlapping. Although the scatter of the points is considerable because only 2 to 3 seeds were used at each load, it can be seen that the values of log S_7 for the three grades lie between 0.46_2 (medium) and 0.485 (fine), and represent a mean of 0.47_2 with a variation of ± 0.010, which corresponds to

$\pm$ 2.5% in S_7. Therefore the effect of changing the grade of the filter paper is only of the order of 2 to 3%. This result is not substantially altered when account is taken of the varying weights of the filter papers, by dividing the above sensitivities by the respective areas for unit weight, see below.

Variation in weight of the filter paper will clearly produce a corresponding change in the sensitivity because the area of the oil spot should be inversely proportional to the mass per unit area of the absorbing material. This effect can be seen clearly by comparing the mean effective S_7 for the Whatman papers, viz. 2.96 ± 0.05, with the figure of 4.10 ± 0.5 for the Greens 401 paper (wt. $= 568 \pm 2$ mg.) at the same humidity. The two are exactly in the inverse ratio of the weights, and a constant figure of 2.35 ± 0.01 is obtained by multiplying the effective S_7 by the weight of one disc in grammes. It is noteworthy in this connection that, whereas a variation of as much as $\pm$ 4% has been noted in the weights of individual filter papers, the means of five (as given above) are constant to within $\pm$ 1%. This points up the importance of using a sandwich of at least four filter discs for one kernel.

6. Optimum Conditions and Determination of Calibration Constant by Comparison with Soxhlet Extraction

On the basis of the foregoing results, we can fix upon the following as optimum conditions for the use of this method of oil estimation, viz.

(i) Diameter of filter discs to be 5 to 11 cm.
(ii) Filter discs to be maintained at a controlled relative humidity, preferably about 80%.
(iii) A sandwich of at least 4 discs to be used for one kernel.
(iv) Crushing Load to be about 5 to 10 tons and "Effective" area of oil spot to be used to eliminate effect of water content of kernel.
(v) The mean weight of the sandwich of filter discs should be recorded if the highest precision is aimed at.

The final step was to determine a constant factor for direct conversion of the area of oil spot measured as above into the oil content of the kernel. This has been done by taking a sample of about 20 g. cottonseeds of a given variety and dividing it into four representative sub-samples by successive "quartering". One of these four sub-samples was used for measuring the mean area of oil spot per mg. of kernel, while another was used for making three determinations of oil content (after dehulling) by the usual soxhlet method. (About 5 to 10% of the kernels were found to be partly shrivelled). The results obtained with cottonseeds of the M 4 variety, using sandwiches of four Green's 401 filter papers (5.5 cm. dia, R. H. = 81%), are given below:

Effective Sensitivity $= 5.10 \pm 0.15$ sq. mm./m.g of kernel (on 10% moisture basis)

Measured oil content $= 27.5\% \pm 1.0\%$ of kernel (on 10% moisutre basis),

that is $= 0.275 \pm 0.010$ mg. per mg. of kernel

$\therefore$ Conversion factor

$$= \frac{\text{Measured oil content per mg. kernel}}{\text{Effective Sensitivity}}$$

$$= \frac{0.275 \pm 0.010}{5.10 \pm 0.15} = 0.054 \pm 0.002 \text{ mg./sq. mm.}$$

Similar determinations were made with a sample of L. S. S. cottonseeds and gave the value

$$\text{Conversion factor} = \frac{0.328 \pm 0.015}{5.60 \pm 0.12}$$

$$= 0.058 \pm 0.002 \text{ mg./sq. mm.},$$

thus yielding a mean factor of 0.056 ± 0.002 mg./sq. mm. for conversion of the "effective" area of oil-spot into the quantity of oil.

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THE RELATIONSHIP BETWEEN VISCOSITY AND MOLECULAR STRUCTURE

Part IV.—Observation of Energy-level Structure in the Intermolecular Potential Energy of Activation for Viscous Flow of Water

MAZHAR MAHMOOD QURASHI AND A. K. M. AHSANULLAH

Physical Research Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

(Received February 22, 1960)

The earlier measurements on the activation energy, ε, of glycerol and ethylene glycol have now been followed up by similar accurate measurements in the case of water, based on the logarithmic derivative of the Andrade equation, viz.,

$$\varepsilon/k = - T^2 \Delta \ln \eta/\Delta T$$

using a Beckmann thermometer for accurate measurement of the 2°C. interval, ΔT. Preliminary experiments in the range of 30°C. to 75°C. have indicated the presence of a series of more or less sharp, regularly occurring steps in the energy, ε. The temperature interval between successive steps is 6°C. on the average, as against 12.1°C. in the case of ethylene glycol. More refined and extensive measurements on water are in hand.

Preliminary viscosity experiments carried out on some mineral oils in this laboratory[1] had indicated a periodicity in the temperature variation of the activation energy, ε, as determined from the equation

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where ε measures the height of the intermolecular potential energy barrier[2,3] that has to be overcome for the relative movement of one layer of molecules with respect to the next (through a distance equal to the length of one molecular aggregate). More accurate measurements of ε at intervals of 5°C. and 2.5°C. on redistilled glycerol[4] and ethylene glycol[5] showed that these liquids have constant ε (and constant A) over certain short, regularly recurring, ranges of temperature (Fig. 1), thus simulating an ideal non-associated liquid in these ranges.

Because of the importance of these previously unrecognized phenomena in the molecular theory of liquids, we have further extended these measurements to the important case of water (twice-distilled), taking readings at intervals of 2°C., with temperature control to better than 0.002°C. The calculations, as in the earlier papers from this laboratory, are based on differentiation of the logarithmic form of equation (1), which yields

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the desired accuracy being attained by measuring the temperature interval, ΔT, with a calibrated Beckmann differential thermometer, and taking viscosity measurements in a U-tube viscometer giving times of flow of the order of 1000 seconds. The results obtained in the range of 30°C. to 75°C. from two separate lots of twice-distilled conductivity water are shown in Table 1 and Fig. 2 (Expt. I and Expt. II), together with the means of the two sets, the radii of the circles being equal to the experimentally estimated standard deviation of the plotted points, which is about $\pm$ 0.009 units of $(\varepsilon/k) \div 1000$.

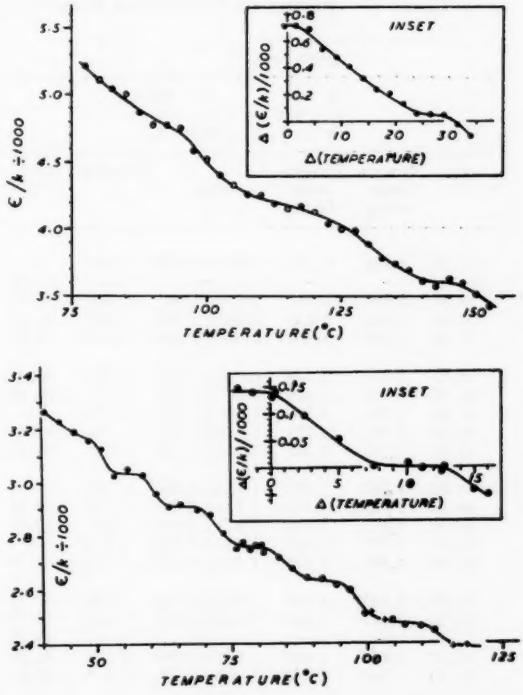

Fig. 1.—Plots showing the previously reported temperature dependence of the activation energy, ε, of viscous flow for (top) glycerol and (bottom) ethylene glycol. The ordinates are $(\varepsilon/k)/1000$, where k is Boltzmann's constant, and the insets show the averaged variation over one segment.

$\pm$ 2.5% in S_7. Therefore the effect of changing the grade of the filter paper is only of the order of 2 to 3%. This result is not substantially altered when account is taken of the varying weights of the filter papers, by dividing the above sensitivities by the respective areas for unit weight, see below.

Variation in weight of the filter paper will clearly produce a corresponding change in the sensitivity because the area of the oil spot should be inversely proportional to the mass per unit area of the absorbing material. This effect can be seen clearly by comparing the mean effective S_7 for the Whatman papers, viz. 2.96 ± 0.05, with the figure of 4.10 ± 0.5 for the Greens 401 paper (wt. $= 568 \pm 2$ mg.) at the same humidity. The two are exactly in the inverse ratio of the weights, and a constant figure of 2.35 ± 0.01 is obtained by multiplying the effective S_7 by the weight of one disc in grammes. It is noteworthy in this connection that, whereas a variation of as much as $\pm$ 4% has been noted in the weights of individual filter papers, the means of five (as given above) are constant to within $\pm$ 1%. This points up the importance of using a sandwich of at least four filter discs for one kernel.

6. Optimum Conditions and Determination of Calibration Constant by Comparison with Soxhlet Extraction

On the basis of the foregoing results, we can fix upon the following as optimum conditions for the use of this method of oil estimation, viz.

(i) Diameter of filter discs to be 5 to 11 cm.
(ii) Filter discs to be maintained at a controlled relative humidity, preferably about 80%.
(iii) A sandwich of at least 4 discs to be used for one kernel.
(iv) Crushing Load to be about 5 to 10 tons and "Effective" area of oil spot to be used to eliminate effect of water content of kernel.
(v) The mean weight of the sandwich of filter discs should be recorded if the highest precision is aimed at.

The final step was to determine a constant factor for direct conversion of the area of oil spot measured as above into the oil content of the kernel. This has been done by taking a sample of about 20 g. cottonseeds of a given variety and dividing it into four representative sub-samples by successive "quartering". One of these four sub-samples was used for measuring the mean area of oil spot per mg. of kernel, while another was used for making three determinations of oil content (after dehulling) by the usual soxhlet method. (About 5 to 10% of the kernels were found to be partly shrivelled). The results obtained with cottonseeds of the M 4 variety, using sandwiches of four Green's 401 filter papers (5.5 cm. dia, R. H. = 81%), are given below:

Effective Sensitivity $= 5.10 \pm 0.15$ sq. mm./m.g of kernel (on 10% moisture basis)

Measured oil content $= 27.5\% \pm 1.0\%$ of kernel (on 10% moisutre basis),

that is $= 0.275 \pm 0.010$ mg. per mg. of kernel

$\therefore$ Conversion factor

$$= \frac{\text{Measured oil content per mg. kernel}}{\text{Effective Sensitivity}}$$

$$= \frac{0.275 \pm 0.010}{5.10 \pm 0.15} = 0.054 \pm 0.002 \text{ mg./sq. mm.}$$

Similar determinations were made with a sample of L. S. S. cottonseeds and gave the value

$$\text{Conversion factor} = \frac{0.328 \pm 0.015}{5.60 \pm 0.12}$$

$$= 0.058 \pm 0.002 \text{ mg./sq. mm.},$$

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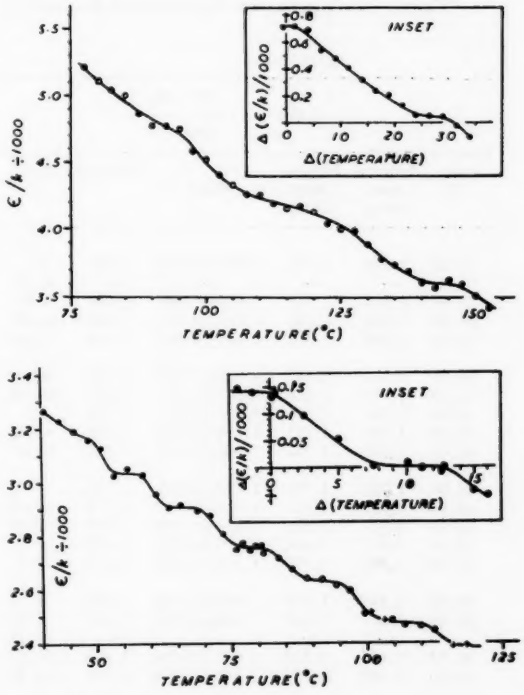

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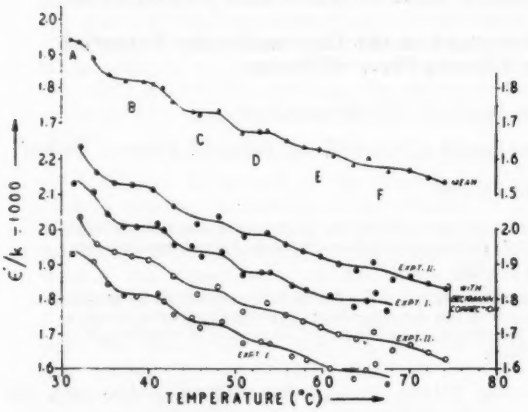

Fig. 2.—Graphs of (ε/k)/1000 obtained from preliminary measurements on water in the range of 30°C. to 75°C. Hollow circles, uncorrected values; solid circles, after application of Beckmann calibration corrections. Successive curves are displaced upwards by 0.1 unit. The triangles (uppermost curve) represent the means of the corrected values from expts. I and II, and the successive steps observed are marked A,B,.....F.

The graphs of Fig. 2 demonstrate the reproducibility of the phenomena under investigation, and show the presence of several flat regions (A , B,.....F) of constant ε, with definite steps between successive "flats". These steps decrease in height with increasing temperature, and are actually much sharper than those seen in Fig. 1, because the drop now extends over just 3°C. on the average, which is only 1°C. more than the experimental measuring interval of 2°C. The energy change, $\Delta\varepsilon$, at each step, which varies from step to step, is of the order of $0.1 \times (1000\ k)$, i.e. 0.009 electron volt, and is considerably lower than the thermal energy, kT. The repetition period of the steps for water is nearly 6°C., as against the corresponding figures of 12°C. and 25°C. in case of ethylene glycol and glycerol, respectively. These intervals are all closely mul-

TABLE 1.—VALUES OF ACTIVATION ENERGY, ε, OBTAINED WITH A MEASURING INTERVAL $\Delta T = 2°C$.

Experiment I					Experiment II					Overall Mean	
	$\frac{\varepsilon}{k}/1000 = -\frac{T^2}{1000}\frac{\Delta \ln \eta_l}{\Delta T}$					$\frac{\varepsilon}{k}/1000 = -\frac{T^2}{1000}\frac{\Delta \ln \eta_l}{\Delta T}$					
Temperature, °C.	Temperature rising	Temperature falling	Mean	Corrected	Temperature, °C.	Temperature rising	Temperature falling	Mean	Corrected	Temperature °C.	$\frac{\varepsilon}{k}/1000$
31.20	1.928	1.932	1.930±0.002	1.930	—	—	—	—	—	31.20	1.930±0.002
—	—	—	—	—	32.19	1.930	1.933	1.932±0.001	1.936	32.19	1.936±0.001
33.65	1.914	1.912	1.913±0.001	1.904	34.07	1.864	1.859	1.862±0.003	1.858	33.86	1.881±0.023
35.25	1.848	1.840	1.844±0.004	1.844	36.10	1.835	1.843	1.839±0.004	1.839	35.67	1.841±0.002
37.06	1.806	1.801	1.804±0.002	1.808	38.08	1.818	1.818	1.818±0.000	1.816	37.57	1.812±0.006
38.63	1.803	1.807	1.805±0.002	1.805	40.48	1.800	1.809	1.804±0.004	1.806	39.55	1.806±0.001
41.10	1.816	1.818	1.817±0.001	1.807	40.48	1.800	1.809	1.804±0.004	1.806	40.79	1.806±0.000
41.80	1.794	1.799	1.796±0.002	1.778	—	—	—	—	—	41.80	1.778±0.002
42.89	1.756	1.751	1.754±0.002	1.758	42.87	1.768	1.769	1.768±0.001	1.760	42.88	1.759±0.001
45.01	1.741	1.744	1.742±0.002	1.742	45.56	1.720	1.716	1.718±0.002	1.721	45.28	1.732±0.011
46.04	1.732	1.732	1.732±0.000	1.732	—	—	—	—	—	46.04	1.732±0.001
48.54	1.732	1.735	1.734±0.002	1.725	48.14	1.733	1.729	1.731±0.002	1.726	48.34	1.726±0.000
50.90	1.672	1.682	1.677±0.005	1.679	50.99	1.671	1.671	1.671±0.000	1.672	50.94	1.676±0.003
52.97	1.677	1.684	1.680±0.004	1.672	—	—	—	—	—	52.97	1.672±0.003
53.95	1.670	1.674	1.672±0.002	1.673	53.70	1.687	1.684	1.686±0.002	1.678	53.82	1.676±0.002
56.50	1.645	1.640	1.642±0.002	1.635	55.79	1.667	1.657	1.662±0.005	1.662	56.14	1.648±0.013
58.20	1.647	1.648	1.648±0.000	1.652	57.80	1.631	1.640	1.636±0.004	1.635	58.00	1.644±0.008
—	—	—	—	—	59.79	1.616	1.618	1.617±0.001	1.616	59.79	1.616±0.001
60.92	1.605	1.612	1.608±0.004	1.603	61.87	1.604	1.606	1.605±0.001	1.598	61.40	1.600±0.002
63.60	1.582	1.573	1.578±0.004	1.578	63.88	1.587	1.588	1.588±0.001	1.588	63.74	1.583±0.005
65.05	1.592	1.599	1.596±0.004	1.596	65.92	1.601	1.603	1.602±0.001	1.595	65.48	1.596±0.004
67.42	1.565	1.574	1.570±0.002	1.566	68.05	1.546	1.547	1.546±0.001	1.550	67.74	1.588±0.008
—	—	—	—	—	70.06	1.573	1.574	1.574±0.001	1.566	70.06	1.566±0.001
—	—	—	—	—	72.24	1.546	1.547	1.546±0.001	1.546	72.24	1.546±0.001
—	—	—	—	—	74.20	1.533	1.534	1.534±0.001	1.526	74.20	1.526±0.001

tiples of 6.2°C., which thus appears to define a basic periodicity for the inter-molecular OH-group interactions common to these three liquids.

The above observations provide evidence for the existence of a well-defined periodic energy structure in the three liquids, probably associated with the molecular aggregation produced by the hydrogen bonds. Further experiments are presently being directed towards obtaining more accurate and extensive data for water, so as to elucidate the detailed structure of the steps, and towards investigating the factors causing the comparatively gradual drop between steps in the case of glycerol and ethylene glycol.

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NOTE ON A FULLY DYNAMICAL VARIANT OF LEES' DISC METHOD FOR THE RAPID DETERMINATION OF THE THERMAL CONDUCTIVITY OF POOR CONDUCTORS

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As an extension of the previously reported semi-dynamical method utilizing the linearity of the plot of $\partial\theta/\partial t$ against θ, the temperature of the standard brass disc, a fully dynamical variant has been developed by extrapolating this linear plot backwards to the initial condition at $t = 0$. The experimental results indicate the necessity for a significant correction for loss of heat from the periphery of the sample disc, an expression for which is derived. The experimental values obtaind from the fully dynamical measurements are shown to be in satisfactory agreement (within $\pm$ 4%) with that obtained by the equilibrium method. The resulting formula contains a small term involving the specific heat of the sample disc, which can thus be determined from two measurements, with a thin disc and a thick one.

1. Basis of the Fully Dynamical Method

In a previous communication,[1] the approach to equilibrium in the measurement of thermal conductivities by Lees' disc method was analyzed mathematically, and for time $t > 0.1\ d/E'_b$, where d is the thickness of the experimental disc and E'_b the effective emissivity of the brass disc, the following equation was derived

$$(m'c' + \tfrac{1}{3}mc)\frac{\partial\theta_d}{\partial t} = \frac{KA}{d}(\theta_s - \theta_d) - A'E'(\theta_d - \theta_{amb}), \quad (1)$$

where m is the mass of the experimental disc, A is its area of cross section and c its thermal capacity, θ_s is the temperature of the steam chest and θ_d that of the brass disc, and m′, c′, A′, E′, all refer to the brass disc (with $A'E' = A \times E'_b$). Experiments carried out on composite discs of different thicknesses (Fig. 1), give straight line plots for $\partial\theta_d/\partial t$ against θ_d in accordance with equation (1).

Another useful result is obtained if we extrapolate the linear portion of the graph in the other direction to $\theta_d = \theta_{amb}$ i.e. towards $t = 0$. Equation (1) then gives

$$(m'c' + \tfrac{1}{3}mc)\,(\partial\theta_d/\partial t)_{t\to 0} = \frac{KA}{d}(\theta_s - \theta_{amb}), \quad (2)$$

and, if we also include the correction term given by the next approximation worked out in the previous paper, this becomes

$$\left\{ m'c' + \frac{1}{3}mc + \frac{1}{45}\frac{(mc)^2}{m'c'} \right\}(\partial\theta_d/\partial t)_{t\to 0} = \frac{KA}{d}(\theta_s - \theta_{amb}),$$

whence

$$K = \frac{d}{A(\theta_s - \theta_{amb})}\left\{ m'c' + \tfrac{1}{3}mc + \frac{1}{45}\frac{(mc)^2}{m'c'} \right\}(\partial\theta_d/\partial t)_{t\to 0}$$

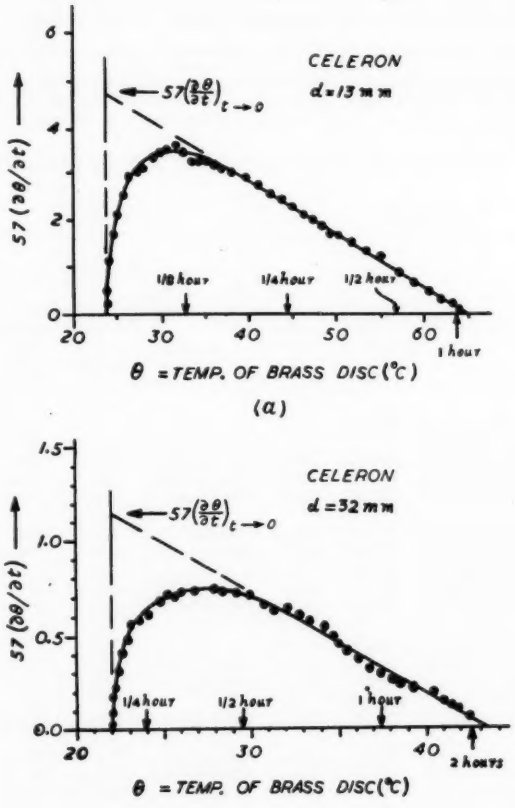

Fig. 1.—Experimental curves for $\partial\theta/\partial t$ against θ, the temperature of the brass disc, obtained with two thickness of "Celeron" discs, namely 13 mm. ($\frac{1}{2}$") and 32 mm. ($1\frac{1}{4}$"). The horizontal arrow indicates the value of 57 $(\partial\theta/\partial t)$ extrapolated linearly to the initial condition ($t = 0$).

$$= \left\{ 1 + \frac{1}{3}\frac{mc}{m'c'} + \frac{1}{45}\left(\frac{mc}{m'c'}\right)^2 \right\} \frac{m'c'd}{A(\theta_s - \theta_{amb})}$$

$$\times \left(\partial\theta_d/\partial t\right)_{t\to 0} \qquad (3)$$

This shows that a measure of the thermal conductivity can be obtained without separately measuring the emissivity of the brass disc, as is normally necessary in Lees' disc method. Of course, the factor

$$\left\{ 1 + \frac{1}{3}\,(mc/m'c') + \frac{1}{45}\,(mc/m'c')^2 \right\}$$

is uncertain to the extent that the specific heat, c, of the sample is not known accurately, but for a comparatively thin sample ($d < 2$ cm.) this uncertainty will be small, perhaps of the order of 2%. Moreover, it has been observed by us that, for the majority of building materials, the specific heat, c, is approximately equal to 0.23 ± 0.06. It follows that

$$mc \backsimeq 0.23 \times \text{mass} \backsimeq \tfrac{1}{4}\,\rho \times \text{Volume},$$

and this value can be used in the above formula without significant error.

The fully dynamical method outlined above is in many respects similar to the transient method described by Beatty, Armstrong, and Schoenborn[2] for rapid measurement of the conductivity of plastics and similar materials in the form of thin discs. However, in their method the metal disc is not allowed to radiate heat to the atmosphere, thus giving an ultimate solution of a type that does not allow of a steady state measurement as is done with the apparatus used in the present experiments, and the time lost before a simple linear formula becomes applicable to the transient flow is several times greater ($t > \frac{1}{4}d^2/K$) than for the method suggested above. The accuracy of both methods appears to be of the same order. Gafner[3] has also described a transient method for measuring the thermal conductivity of rocks and building materials, but he uses a heat sink, whose temperature is maintained a fixed number of degrees below that of the source. He claims an accuracy of $\pm$ 7%. The method described in the present paper can be said to be intermediate between the other two, because the metal disc automatically attains a limiting temperature intermediate between that of the heat source and the ambient temperature. The accuracy of the measurement attainable by this method is seen in the next section to be of the order of $\pm$ 4%.

2. Some Experimental Results

In order to examine in detail the applicability of equation (3), we define a quantity "$K_{dynamic}$" as

$$K_{dynamic} = \frac{d\,m'\,c'}{A(\theta_s - \theta_{amb})}\left(\partial\theta_d/\partial t\right)_{t\to 0}$$

$$= K \Big/ \left\{ 1 + \frac{1}{3}\frac{mc}{m'c'} + \frac{1}{45}\left(\frac{mc}{m'c'}\right)^2 \right\} \qquad (4)$$

Then, if the value of K determined from the equilibrium temperature of the brass disc is called "$K_{equilib}$", we have $K = K_{equilib}$ for a thin disc, and get, from equation (4),

$$K_{equilib}/K_{dynamic} = 1 + \frac{1}{3}\frac{mc}{m'c'} + \frac{1}{45}\left(\frac{mc}{m'c'}\right)^2$$

$$\backsimeq 1 + d \times \text{Constant}, \qquad (5a)$$

because for a given material, m c is proportional to the sample thickness, d. If we take the reciprocal, we get

$$K_{dynamic}/K_{equilib} = 1 - \frac{1}{3}\frac{mc}{m'c'} + \frac{4}{45}\left(\frac{mc}{m'c'}\right)$$

$$+ \ldots \backsimeq 1 - d \times \text{Constant}. \qquad (5b)$$

Table 1 gives values of both the above ratios determined experimentally for values of d ranging from 1/4 inch up to 7/4 inches for discs of "Celeron", a laminated insulating material, typical graphs of $\partial\theta/\partial t$ against θ for which have already been shown in Fig. 1.

A graph of either of the two above ratios when plotted against 'd' should be nearly linear (the approximation being expected to be better for the first ratio, $K_{dynamic}/K_{equilib}$), and should extrapolate to unity for $d = 0$. Figures 2(a) and 2(b) show the experimental data of Table 1 plotted according to equations (5a) and (5b) respectively. (The measurements with the 1/4 inch thickness of celeron are given one third the weight of the others, the corresponding value of K determined by the equilibrium method being abnormally low, probably because the temperature of the brass disc becomes high enough (80°C.) to make the use of Newton's law somewhat erroneous). Both graphs do extrapolate to unity within the limits of experimental error, and the standard deviation of the experimental points indicates an accuracy of about $\pm$ 4%, cf. the standard deviation of 0.034 for the values of $K_{dynamic}/K_{equilib}$ in Table 1.

3. Influence of Peripheral Heat Loss

An interesting feature of the graphs of Fig. 2

is that, contrary to expectation, linearity is nearly exact in the case of equation (5b), while there is a very marked curvature for large values of d when ($K_{equilib}/K_{dynamic}$) is plotted against d. This discrepancy is largely due to the effect of the heat lost from the peripheral surface of the specimen, together with a contribution from the errors remaining in the second approximation for the derivation of equation 2 (cf equation 3). The peripheral heat loss is the more important factor, because it alters the first order terms in equations (5). This heat loss occurs in the equilibrium measurement as well as in the dynamical measurement, but the value of the correction factor for this effect is different in the two cases. The correction factor for the measurement by the equilibrium method was derived in a previous paper[4] (equation (14)), and the formula is

$$K_{equilib}/K = \left[1 - \frac{E}{K}\frac{d}{R} \times \left\{ \frac{d}{2} + d \quad (\theta_d - \theta_{amb})/(\theta_s - \theta_{amb}) \right\} \right] \qquad (6a)$$

$$= 1 - \frac{E}{K}\frac{d}{R}\left(\frac{d}{2} + K_{equilib}/E'_b \right),$$

which can be recast to give

$$K_{equllib}/K = \left(1 - \frac{E}{K}\frac{d^2}{2R} \right) \div \left(1 + \frac{E}{K}\frac{d}{R} \times \frac{K}{E'_b} \right), \qquad (6b)$$

where E is the emissivity of the sample and K is the actual value of the thermal conductivity. An estimate of the corresponding factor for the measurement by the fully dynamical method under discussion can be obtained by the following simple argument.

An examination of the analysis[1] leading upto equation (1) describing the linear part of the graphs for $\partial\theta_d/\partial t$ against θ_d (cf. Fig. 1) shows that this linear part corresponds to the region in which the temperature distribution across the thickness of the sample disc can also be treated as linear (with the inclusion of a small second-order term). Thus, in this region, the relationship of the peripheral loss to the conduction flow of heat will be similar to that under the equilibrium condition, provided that due account is taken of the different temperature of the the disc. Therefore, for

TABLE 1.—COMPARISON OF MEASUREMENT BY EQUILIBRIUM AND FULLY DYNAMICAL METHODS (EXPERIMENTAL RESULTS FROM TWO SETS OF EXPERIMENTS).

Thickness of disc. (in.)	$K_{equilib} \times 10^3$ (cal. cm.$^{-1}$ sec.$^{-1}$/°C.)		$K_{dynamic} \times 10^3$ (cal. cm.$^{-1}$ sec.$^{-1}$/°C.)		$\frac{K_{equilib}}{K_{dynamic}}$	$\frac{K_{dynamic}}{K_{equilib}}$
1/4	0.383 0.387	0.385 ±.002	0.350 0.330	0.340 ±.010	$1.13_2 \pm .03$	$0.88_4 \pm 0.027$
1/2	0.406 0.423	0.414 ±.008	0.374 0.386	0.380 ±.006	$1.09_0 \pm .03$	$0.91_7 \pm 0.023$
3/4	0.396 0.392	0.394 ±.002	0.326 0.332	0.329 ±.003	$1.19_7 \pm .01$	$0.83_5 \pm 0.009$
1	0.384 0.359	0.372 ±.012	0.276 0.268	0.272 ±.004	$1.36_7 \pm .05$	$0.73_2 \pm 0.026$
5/4	0.347 0.318	0.332 ±.014	0.242 0.254	0.248 ±.006	$1.33_8 \pm .06$	$0.74_7 \pm 0.036$
3/2	0.313 0.316	0.314 ±.002	0.182 0.207	0.194_5 ±.013	$1.61_5 \pm .10$	0.619 ± 0.041
7/4	0.290	0.290	0.160	0.160	1.81	0.55_2
	Std. Dev. =0.009		Std. Dev. = 0.008			Std. Dev. = 0.034

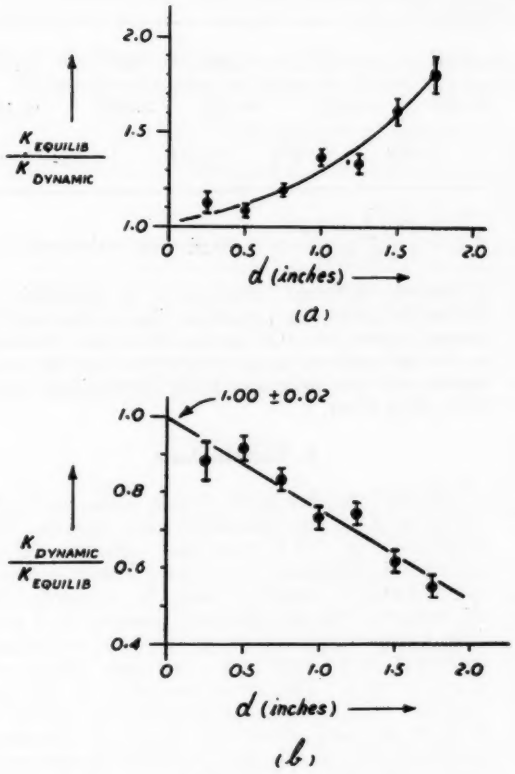

Fig. 2.—Plots of the ratios (a) $K_{equilib}/K_{dynamic}$ and (b) $K_{dynamic}/K_{equilib}$ as determined for different thicknesses of "Celeron", showing the nearly linear variation in the case of (b).

any intermediate position on the linear part of the $\partial\theta/\partial t$ against θ graph, say at a temperature θ'_d of the brass disc, the correction factor will be given by the expression (6a), but with θ'_d written in place of the equilibrium value θ_d. When the linear part of the graph is extrapolated (linearly) to $t = 0$ for the calculation of $K_{dynamical}$, then θ'_d becomes equal to θ_{amb}, the ambient temperature, and therefore from equation (6a) the correction factor for the peripheral loss simply reduces to

$$\left\{1 - \frac{E}{K}\frac{d}{R}\left(\frac{d}{2} + 0\right)\right\} = \left(1 - \frac{E}{K}\frac{d^2}{2R}\right). \tag{6c}$$

Introduction of this correcting factor into equation (4) gives us

$$K_{dynamic} = K\left(1 - \frac{E}{K}\frac{d^2}{2R}\right)$$

$$\div \left\{1 + \frac{1}{3}\frac{mc}{m'c'} + \frac{1}{45}\left(\frac{mc}{m'c'}\right)^2\right\} \tag{7}$$

$$= K_{equilib}\left(1 + \frac{E}{K}\frac{d}{R}\frac{K}{E'_b}\right)$$

$$\div \left\{1 + \frac{1}{3}\frac{mc}{m'c'} + \frac{1}{45}\left(\frac{mc}{m'c'}\right)^2\right\},$$

whence

$$K_{dynamic}/K_{equilib} = \left(1 + \frac{d}{R}\frac{E}{E'_b}\right)$$

$$\div \left\{1 + \frac{1}{3}\frac{mc}{m'c'} + \frac{1}{45}\left(\frac{mc}{m'c'}\right)^2\right\} \tag{8a}$$

$$\simeq 1 - \left(\frac{1}{3}\frac{mc}{m'c'} - \frac{d}{R}\times\frac{E}{E'_b}\right)$$

$$+\frac{1}{3}\frac{mc}{m'c'}\left(\frac{4}{15}\frac{mc}{m'c'} - \frac{d}{R}\frac{E}{E'_b}\right). \tag{8b}$$

This equation shows that (i) the slope of the graph for $(K_{dynamic}/K_{equilib})$ against 'd' is considerably less than that given by the approximate equations (5), and (ii) the second-order term, proportional to d^2, also becomes small due to the mutual cancellation of the quantities within the brackets.

4. Indirect Estimation of the Thermal Capacity of Celeron

A particular advantage of the dynamical method is that by using the measurements on samples of two different thicknesses (cf. equation 7), or by combination with the equilibrium measurement (cf. equations 8), the thermal capacity of the material can be found. Let us compare the mean slope of the graph with the theoretical value from equation (8b), which gives, on putting $mc = \rho Ad \times c$,

$$-(\text{Mean Slope}) \simeq \left(\frac{1}{3}\frac{\rho Ac}{m'c'} - \frac{1}{R}\frac{E}{E'_b}\right)$$

$$\div \left(1 + \frac{1}{2}\frac{\rho Ac}{m'c'}\bar{d}\right),$$

whence

$$c\left(1 + \frac{3}{2}\bar{d} \times \text{Slope}\right)$$

$$= \frac{3m'c'}{\rho A}\left(\frac{1}{R}\frac{E}{E'_b} - \text{Slope}\right), \tag{9}$$

TABLE 2

Thickness of disc:	1/4"	1/2"	3/4"	1"	5/4"	3/2"	7/4"
Kx10³ from equation 7:	(0.390)	0.498	0.496	0.473	0.499	0.458	0.446
Mean of successive values		0.46_2	0.49_7	0.48_4	0.48_6	0.47_8	0.45_2

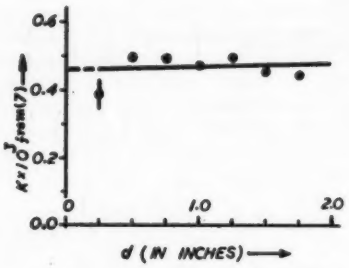

Fig. 3.—Graph showing the residual variation in the corrected value of 'K' as calculated from the fully dynamical measurements using equation (7).

Now the experimental value of the slope is $-\frac{0.48 \pm 0.06}{5}$ i.e. -0.096 ± 0.012 cm.$^{-1}$, while the other constants are[4]

$$\begin{aligned} m'c' &= 45.9 \text{ cal./g./°C.,} \\ A &= 76 \text{ sq. cm.} \\ R &= 5\text{cm.} \\ E/E'_b &= 0.053/0.29 = 0.18 \\ \rho &= \text{density of "Celeron"} \\ &= 1.30 \text{ g./cc.} \end{aligned}$$

It follows that the specific heat 'c' of the celeron is given by

$$\left(1 - \frac{3}{2} \times 2.54 \times .096\right) \times c = \frac{3 \times 45.9}{1.3 \times 76}$$

$$\times \left(\frac{0.18}{5} + 0.096\right) = 1.40 \times (0.036 + 0.096)$$

$$= 0.18_5 \pm 0.02,$$

whence

$$c = \frac{0.185 \pm 0.02}{1 - 0.366} = 0.29 \pm 0.03 \text{ calories/g./°C.}$$

A slightly different value of 'c' is obtained by fitting the more exact equation (8a) to the experimental curve at 1.6 inches thickness, yielding $c = 0.28$, and the mean of the two is to be compared with the calorimetrically determined value of 0.33 ± 0.03.

5. Conclusions

Finally, we give in the Table 2 the corrected values of $K \times 10^3$ calculated from equation (7) with $c = 0.30$. It is seen that equation (7) is not entirely adequate, there being a small second-order residual variation with d. Nevertheless, the value of K extrapolated linearly to $d = 0$ (Fig. 3) comes out to $(0.46 \pm 0.01) \times 10^{-3}$, which compares well with the previous figure[4] of 0.450×10^{-3}. The mean variation of the individual values about the straight line of Fig. 3 is ± 0.025 and about the means of successive pairs in Table 2 is only ± 0.015 (when the measurement with the 1/4" disc is given one-half the weight of the others), thus indicating an accuracy of about $\pm 5\%$.

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STUDIES IN THE RELATIONSHIP BETWEEN VISCOSITY AND MOLECULAR STRUCTURE

Part V.—Preliminary Investigation of Periodicity in the Temperature Variation of the Activation Energy in Aqueous Ethyl Alcohol in the Temperature Range of 5°C. to 55°C.

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As a sequel to the discovery of well-defined steps in the temperature variation of inter-molecular activation energy of viscous flow in poly-hydroxylic liquids like glycerol, ethylene glycol and water, the investigations have been extended to the case of ethyl alcohol, which has one hydroxyl group. Preliminary results obtained with a 2°C. measuring interval are reported here, and they show the presence of a fairly regular sinusoidal variation with a period of 10.4°C. and a peak-to-peak amplitude of about 0.102 units of $(\varepsilon/k)/1000$ for redistilled rectified spirits (ethyl alcohol containing 6.5% water). Similar experiments on ethyl alcohol containing 12.3% water indicate a much smaller variation of peak-to-peak amplitude nearly 0.02 units with a period of about 8.8°C. These results are generally compatible with the extensive measurements already existing on the water-ethyl alcohol system, but the standard data is not accurate enough to indicate the small variations described in the present communication.

Introduction

The existence of a temperature periodicity in the intermolecular activation energy of viscous flow has been investigated in this laboratory for several poly-hydroxylic liquids, namely glycerol, ethylene glycol and water, and described in previous communications[1–3] of this series. It was found that in all cases the experiments showed the existence of regularly recurring ranges of temperature, within which the activation energy is exactly constant. The drop from one step to the next is not, however, equally sharp for all cases. Since the liquids examined so far have two or three hydroxyl groups in the molecule, it was considered worthwhile to carry out similar investigations with some mono-hydroxylic liquids. In the present communication, the status of the existing standard data on ethyl alcohol is examined, and a preliminary account is given of more accurate results obtained with aqueous ethyl alcohol in the relatively easily accessible temperature range of 5°C. to 55°C. The experiments with absolute alcohol will be reported separately.

1. Consideration of Earlier Standard Data

Standard data on the viscosity of ethyl alcohol at several temperatures are available in Kaye and Laby's *Tables of Physical and Chemical Constants*[4] and in *Physico-Chemical Constants of Pure Organic Compounds*[5] (data of Titani (1927) and others). These data, which are compiled from the work of several authors, are believed to be accurate to somewhere around ± 0.001 centipoise. The data from Kaye and Laby are shown in the second row of Table 1, while the third row gives the calculated values of $\Delta \ln \eta/\Delta T$ and the fourth row shows the corresponding values of the quantity $(\varepsilon/k)/1000$ calculated by using the well known logarithmic derivative of the Andrade equation, viz.

$$-\frac{\Delta \ln \eta}{\Delta T} = \frac{\varepsilon}{k}/T^2, \qquad (1)$$

where 'ε' is the activation energy of viscous flow for the liquid and 'k' is Boltzmann's constant. In the present case, ΔT is limited to being 10°C., and accordingly for intermediate temperatures the interpolated values of $(\varepsilon/k)/1000$ at 10°C., 20°C., etc. are given in parentheses. On the basis of an accuracy of ± 0.001 centipoise in the values of viscosity, the average uncertainty in the calculated values of $(\varepsilon/k)/1000$ works out to be of the order of ± 0.02. The above values of ε/k are plotted as hollow circles in Fig. 1(a) (top), while the corresponding values calculated from the set of standard data in *Physico-Chemical Constants of Pure Organic Compounds* are plotted as crosses in Fig. 1(a) (top), and the smoothed values from the graph are given in the fifth row of Table 1. Although both graphs show a prominent maximum followed by a minimum, the discrepancies between the two sets of values are very considerable, especially in the region of 20°C. to 35°C., and the crosses are, on the average, higher by about 0.05 units.

In the next row of Table 1 are given the means of the two sets of values, together with their standard errors, the sign ± indicating that the value in the second set is higher than that in the first set. The average standard error of the means comes out to be ± (0.022 ± 0.046), indicating that the truly random standard error is 0.046, while there is a consistent discrepancy of $2 \times 0.022 = 0.044$ between the two sets of values. The mean values of $(\varepsilon/k)/1000$ are plotted in Fig. 1(b) (bottom) as

solid circles with the short vertical lines corresponding to the standard error of 0.046 units estimated above. The straight line shown in the graph fits the plotted points satisfactorily, the maximum deviation being 2.2 times the standard error. There is thus little justification for drawing the dotted curved line through them, which would show a periodicity of about 35°C., with a maximum in the neighbourhood of 32°C. Similar considerations apply to the values of ε/k for alcohol containing 5% and 10% water obtained from the *International Critical Tables*,[6] Vol. V, shown in the last two rows of Table 1, and plotted (squares and triangles) in Fig. 1(b).

It must be noted here for comparison that the heights of the steps observed in previous experiments on ethylene glycol[1,2] and water[3] are of the order of 0.1 unit of $(\varepsilon/k)/1000$, and it thus follows from the above analysis that the standard data plotted in Fig. 1 are quite inadequate for discovering any effect of this order in the case of ethyl alcohol. Therefore the preliminary experiments described herein were undertaken, in which the values of $(\varepsilon/k)/1000$ were directly measured in the range of 5°C. to 55°C., aiming at an accuracy of $\pm$ 0.01 units of $(\varepsilon/k)/1000$. These experiments were first carried out on redistilled rectified spirits with a water-content of about 6%, and were then repeated with alcohol containing 11% water.

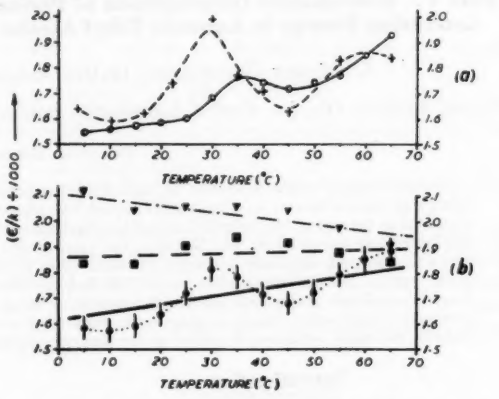

Fig. 1.—(a). Plots of $(\varepsilon/k)/1000$ for ethyl alcohol, as calculated from two sets of standard data (i) Titani and others, crosses and (ii) International Kaye and Laby Tables, hollow circles. (b). Plots of $(\varepsilon/k)/1000$ as calculated from the standard data for the water ethyl alcohol system: (i) solid circles, mean for pure ethyl alcohol, with vertical lines indicating estimated standard deviation, (ii) squares, for alcohol containing 5% water, (iii) triangles, for alcohol with 10% water.

TABLE 1.—INTER-MOLECULAR ACTIVATION ENERGY ε FOR ETHYL ALCOHOL, CALCULATED FROM THE STANDARD PUBLISHED VISCOSITY DATA.

Temperature (°C)	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70
η (cP) Kaye & Laby	1.767	—	1.447	—	1.197	—	1.000	—	0.830	—	0.700	—	0.594	—	0.502
$-100(\Delta \ln \eta/\Delta T)$	—	2.00_0	—	1.89_6	—	1.80_0	—	1.86_2	—	1.69_6	—	1.64_2	—	1.68_4	
$\frac{\varepsilon/k}{1000} = \frac{T^2}{1000} \times (\Delta \ln \eta/\Delta T)$	—	1.54_6	(1.56_0)	1.57_5	(1.58_8)	1.60_2	(1.68_5)	1.76_8	(1.74_4)	1.72_0	(1.74_5)	1.77_0	(1.85_1)	1.93_2	
$\frac{\varepsilon/k}{1000}$ Calculated from "Physico-Chemical Constants of Organic Compounds."	1.624	1.59_1	1.60_8	1.68_8	1.84_0	1.94_5	1.80_2	1.69_0	1.64_2	1.71_2	1.818	1.86_0	1.84_0		
Mean $\frac{\varepsilon/k}{1000}$	1.58_5	1.57_6	1.59_2	1.63_8	1.72_1	1.81_5	1.78_5	1.71_7	1.68_1	1.72_8	1.79_4	1.85_6	1.88_6		
	$\pm.039$	.016	$\pm.016$	$\pm.050$	$\pm.119$	$\pm.130$	$\pm.017$	$\pm.027$	$\pm.039$	$\pm.016$	$\pm.024$	$\pm.004$	$\pm.046$		
$\frac{\varepsilon/k}{1000}$ with 5% water (Int. Crit. Tables)	1.83_8	—	1.83_6	—	1.90_5	—	1.94_0	—	1.91_6	—	1.88_2	—	1.84_5		
$\frac{\varepsilon/k}{1000}$ with 10% water (Int. Crit. Tables)	2.12_0	—	2.04_2	—	2.05_8	—	2.06_2	—	2.04_5	—	1.97_8	—	1.90_2		

2. Details of Experimental Procedure

The apparatus was essentially the same as that used previously for the investigations on water,[3] including the ballast bottle device for controlling the evaporation of the experimental liquid from the viscometer. The measurement of the level of the liquid with reference to the fiducial mark above the large lower bulb of the U-tube viscometer was however carried out by mounting a vertical transparent millimetre scale immediately behind the viscometer, both the scale and the fiducial mark being observed simultaneously through a cathetometer telescope placed about eight feet away. Readings of level could thus be taken to 0.1 mm. or a little better, which suffices to give an accuracy of 0.01% in the corrected flow times.

U-tube Viscometer No. 1 (B. S. S.) with a constant of 0.004,25 was used in these preliminary experiments, and it gave flow times ranging from 350 secs. at 10°C., to 150 secs. at 55°C. Since three to five measurements are taken at each temperature, an accuracy of 0.02 secs. is attainable in the flow time, which, for a measuring interval Δ T of 2°C., leads to a reliability of about $\pm$0.4% in the value of the energy ε calculated by means of the formula (1), which can be put as

$$\begin{aligned} \varepsilon/k &= -\,T^2\,\Delta\,\ln\eta/\Delta T \\ &= -\,T^2\Delta\ln\nu/\Delta T - \frac{T^2}{\rho}\,\Delta\rho/\Delta T \\ &= \varepsilon'/k - \frac{T^2}{\rho}\,\Delta\rho/\Delta T, \end{aligned} \qquad (2)$$

where the second term constitutes a small slow-varying correction. This estimate of 0.4% corresponds to an error of $\pm$ 0.008 in the calculated quantity $\frac{\varepsilon'/k}{1000}$, which itself is of the order of 2 in the range of temperatures studied here.

The ethyl alcohol used for the experiments was purified by repeated distillation, the rectified spirits thus collected containing about 5% water. The water content of the samples prepared for the measurement of activation energy was estimated in two different ways, (i) by density measurements before and after each experiment, and (ii) from the absolute value of viscosity measured at a standard temperature during the experiment itself. The two sets of estimates were in essential agreement with each other.

The experiments were split into two parts, the first from room temperature (which was 25°C. to 30°C.) down to 5°C., and then the second from room temperature up to 55°C. The viscosity measurements were made at intervals of 2°C., and the temperature for each reading was maintained by electrical relays to within $\pm$ 0.005°C.

The temperature measurements were made with an accuracy of $\pm$ 0.005°C., using a calibrated fifth degree thermometer with a magnifying thermometer reader. In this way, an accuracy of about 0.5% could be obtained in the measurements of the two-degree temperature intervals Δ T. This would correspond to an error of $\pm$0.010 in the experimental values of (ε/k)/1000.

3. Experimental Results with Rectified Spirits (containing 6% water)

The results of the first series of experiments with redistilled rectified spirits, extending from 16°C. to 54°C. and corrected for level variation, are given in the first two columns of Table 2, and are plotted in the lowest curve (solid triangles) of Fig. 2, where the short vertical lines indicate the averaged standard deviations estimated experimentally from the differences between the readings obtained with rising and falling temperature sequences. Although these deviations are considerably larger than the expected value of about 0.015, a satisfactory curve can nevertheless be drawn through the experimental points. This curve shows four pairs of alternate maxima and minima with a complete cycle extending over 10° to 12°C. The peak-to-peak amplitude appears to be somewhat variable, and lies between 0.08 and 0.16 units of (ε/k)/1000, but it is probable that part of this variation is due to the rather large standard deviation (about 0.03) of the individual points. (Although it is not easy to fix wholly the precise cause of this large standard

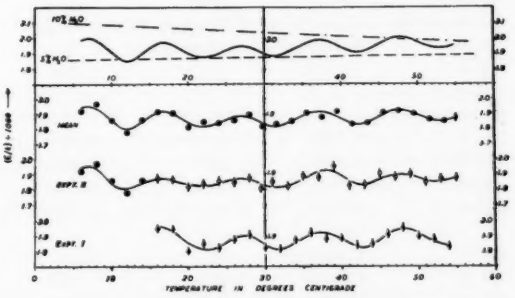

Fig. 2.—(Lower half) Plots of experimental values of (ε/k)/1000 for alcohol having 6.1% water. The short vertical lines indicate the estimated standard deviations of the plotted points for the two separate experiments, while the radii of the solid circles show the standard deviations of the mean values. (Top half) Corrected mean curve for (ε/k)/1000 (full line), compared with the graphs based on earlier standard data for alcohol with 5% water (broken line) and 10% water (chain-dotted line).

deviation, it may be noted for comparison that an average value of ± 0.026 was obtained in the comparable preliminary measurements with ethylene glycol reported in Part II of the present series of communications).[1] The water content of the alcohol, as estimated from its density and value of its viscosity, was found to vary from 5.9% at the beginning of the experiment to 6. 5% at the end, giving a mean of 6.2% ± 0.3%.

A second series of experiments was now carried out with a fresh sample of alcohol (water content from 5.6% to 6.4%), and, in addition to the temperature range previously covered, the measurements were taken down to 5°C., which is the lowest temperature easily attainable by circulating ice-cold water. This enables us to cover an additional complete cycle of variation of the energy ε in the low temperature region. The measurements in the region were taken twice so that the means would be comparable in accuracy with the means of the two series of experiments above 16°C. The results of this second series are given in the third and fourth columns of Table 2, and are

TABLE 2.—PRELIMINARY MEASUREMENTS OF ACTIVATION ENERGY OF VISCOUS FLOW ε FOR RECTIFIED SPIRITS FROM 5°C. TO 55°C.

Experiment I		Experiment II		Mean of I and II		Correction
Temperature (°C.)	$\frac{\varepsilon'/k}{1000} = -T^2\,\Delta\ln\nu/\Delta T \div 1000$	Temperature (°C.)	$\frac{\varepsilon'/k}{1000} = -T^2\,\Delta\ln\nu/\Delta T \div 1000$	Temperature (°C.)	$\frac{\varepsilon'/k}{1000} = -T^2\,\Delta\ln\nu/\Delta T \div 1000$	$T^2\beta/1000$ + Residual thermometric correction
—	—	6.0	1.917 ± .010	6.0	1.917 ± .010	
—	—	8.0	1.965 ± .020	8.0	1.965 ± .020	
—	—	10.0	1.854 ± .016	10.0	1.854 ± .016	0.083 −0.03
—	—	12.0	1.776 ± .011	12.0	1.776 ± .011	
—	—	14.0	1.859 ± .001	14.0	1.859 ± .001	
16.0	1.950	16.0	1.875	16.0	1.912 ± .038	
18.0	1.950	18.0	1.865	18.0	1.908 ± .042	
20.0	1.801	20.0	1.818	20.0	1.810 ∓ .008	0.091 −0.03
22.0	1.848	22.0	1.939	22.0	1.844 ± .005	
24.0	1.824	24.0	1.856	24.0	1.840 ∓ .016	
26.0	1.882	26.0	1.847	26.0	1.864 ± .018	
28.0	1.915	28.0	1.877	28.0	1.896 ± .019	
30.0	1.821	29.5	1.807	29.8	1.814 ± .007	0.101 −0.03
32.0	1.815	31.0	1.851	31.5	1.833 ∓ .018	
34.0	1.972	33.0	1.820	33.5	1.846 ± .026	
36.0	1.920	35.0	1.890	35.5	1.905 ± .015	
38.0	1.881	37.0	1.880	37.5	1.880 ± .000	
40.0	1.886	39.0	1.954	39.5	1.920 ∓ .035	0.112 −0.03
42.0	1.834	41.0	1.829	41.5	1.832 ± .003	
44.0	1.850	43.0	1.832	43.5	1.841 ± .009	
46.0	1.910	45.0	1.905	45.5	1.908 ± .003	
48.0	1.955	47.0	1.882	47.5	1.918 ± .036	
50.0	1.899	49.0	1.904	49.5	1.902 ∓ .002	0.121 −0.03
52.0	1.878	51.0	1.849	51.5	1.864 ± .015	
54.0	1.836	53.0	1.872	53.5	1.854 ∓ .018	
—	—	55.0	1.876	55.0	1.88	

Mean dev. = ± (.006 ± .017)

plotted as hollow circles in Fig. 2 (solid circles below 16°C.). It is seen, after allowing for random errors of the order of 0.03 units of $(\varepsilon/k)/1000$, as previously estimated, that the two graphs are in essential agreement, both as regards the temperatures at which the various maxima and minima occur and as regards the mean amplitude.

4. Discussion of Results with Rectified Spirits

The means of the two series of measurements are shown in the next two columns of Table 2 together with their deviations, where $\mp$ indicates that the values from experiment II are higher. An analysis yields an overall standard deviation of $\pm$ 0.017 for the means as shown in the end of Table 2. These means are plotted as solid circles in Fig. 2, and it is to be noted that the minimum and maximum values of the peak-to-peak amplitude in this mean graph are 0.070 and 0.150, respectively. The average value of the peak-to-peak amplitude for the two complete waves above 30°C. comes out to be 0.088 $\pm$ 0.005, while that for the two waves below this temperature is 0.115 $\pm$ 0.015. Although the difference between these two figures may be indicative of a progressive increase of amplitude with falling temperature, they can be considered equal within the present limits of experimental error, and we may take the mean peak-to-peak amplitude from 5° to 55°C. to be 0.102 $\pm$ 0.012.

In order to examine the degree of uniformity of the temperature interval per cycle, it is convenient to determine the temperature corresponding to the midpoints between each maximum and the neighbouring minimum. These temperatures are arranged in Table 2(a), together with their first differences. The differences range from 4.8° to 5.7°C., thus showing that the temperature interval for each half cycle is reasonably constant. The overall mean value is 5.18° $\pm$ 0.12°C., whence we obtain the mean period of one complete cycle to be twice this, i.e. 10.4° $\pm$ 0.2°C.

For comparison with the broken line graph of Fig. 1(b), based on earlier standard data for alcohol containing 5% water, it is necessary to add the small correction term, $T^2\beta/1000 = -T^2/\rho(\Delta\rho/\Delta T)$ $\div 1000$. Values of this, together with a residual thermometric correction amounting to 0.030, are given in Table 2 (last column). This correction varies slowly, from 0.05 to 0.10, over the whole range of temperatures, and its incorporation will not significantly affect the foregoing quantitative conclusions ragarding the cyclic variation observed in the activation energy. The corrected curve is shown by the full line graph at the top of Fig. 2, while the broken line and the chain dotted line show for comparison the corresponding graphs for alcohol containing 5% and 10% water, respectively, based on the previous standard data as given in Table 1 and Fig. 1(b).

The full line curve (at the top of Fig. 2) lies between the broken and chain dotted lines, and, in the region below 40°C., it would be evenly placed about the previous standard data for ethyl alcohol containing 6.5% water, in satisfactory agreement with the known mean water content of 6.1% $\pm$ 0.4% for our experimental samples of rectified spirits. Above 40°C., the curve obtained from the present experiments is higher by about 0.05 units than the previous standard data for alcohol with 6.5% water. This discrepancy may be significant, but is nevertheless within the limits of error for the previous standard data, cf. Table 1 and Fig. 1. It can thus be concluded that the present measurements are consistent with earlier standard data, but indicate, in addition, the presence of a cyclic variation with a period of 10.4° $\pm$ 0.2°C., and a peak-to- peak amplitude of 0.102 $\pm$ 0.012 units of $(\varepsilon/k) \div 1000$. It is to be noted here that, as shown (both experimentally and theoretically) in earlier papers,[1,2] there will be an exactly corresponding cyclic variation in ln 1/A, with a peak-to-peak amplitude of $(1/T) \times 0.102 \times 1000 = 0.33$, which yields a ratio of 1.4 for A_{max}/A_{min}.

5. Experimental Results with Alcohol containing 12% Water

In order to form some idea of the possible changes with water percentage to be expected in the above characteristics of the curves for ε/k against temperature in the alcohol-water system, another series of experiments was performed with alcohol containing about 12% water. This series

TABLE 2(a)

Temperature at midpoint of max. and min. (°C.) ..	9.2	14.2	19.0	24.4	29.2	34.2	39.8	44.9	50.6
First differences (°C.) ..	5.0	4.8	5.4	4.8	5.0	5.6	5.1	5.7	

is of particular interest because the graphs of Fig. 2 and Fig. 1(b) suggest that some concentration between 6% and 12% water will give an almost constant value of ε/k over the whole temperature range from 0°C. to 70°C. The experimental samples were prepared by adding calculated amounts of double-distilled water to the previously dstilled rectified spirits, and the total water content was estimated by the two methods, as before.

A synopsis of the final results obtained from experiments with three samples of this aqueous alcohol is given in Table 3, which shows the mean of three series of experiments, each with a newly blended sample of the aqueous alcohol, the water contents of the three samples being 12.5 ± 0.7%, 12.2 ± 0.2%, and 12.3 ± 0.8% with an overall mean of 12.3 ± 0.5%.

Two facts are immediately noticeable in Table 3. First, that the values of (ε′/k) ÷ 1000 show an overall decrease of just about 0.1 in the 44 degree interval from 10°C. to 54°C., and second, that the cyclic variation with a period of about 10°C., so clearly observed in the samples with 6% water has apparently disappeard. On plotting the data of Table 3 (on an expanded scale for ε′/k), we get Fig. 3, which shows graphs for ε′/k ÷ 1000 and for the corrected values, together with the graphs based on previous standard data for alcohol with 10% and 12.5% H_2O. It is seen (Fig. 3, lower half) that a small periodic variation with a peak-to-peak amplitude of 0.02 units of (ε′/k) ÷ 1000 can be traced, even though this amplitude is only twice as great as the standard error of the individual values of Table 3. An estimate of the period of this variation can also be made, and is found to be nearly 8.8°C., as against 10.2°C. observed for the samples containing 6% water.

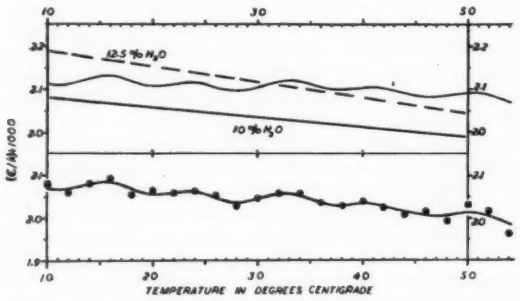

Fig. 3.—(Lower half) Plot of values of (ε/k)/1000 for alcohol containing 12.3% water, obtained as the mean of three separate experiments; the radii of the circles indicate the estimated standard deviation. (Top half) The corrected values of (ε/k)/1000 compared with the graphs based on previous standard data for alcohol containing 10% water (full line) and 12.5% water (broken line).

6. Conclusions

These observations suggest that this period changes smoothly from the already known value of 6.2°C. for water[3] to a value somewhat greater than 10°C., perhaps 2 × 6.2° = 12.4°C., for pure anhydrous ethyl alcohol. On this hypothesis of a gradual transition, the sinusoidal behaviour observed in ethyl alcohol should, with increasing water-content, gradually give place to the stepwise behaviour observed in the case of pure water, and the corrected graph of Fig. 3 (upper half) suggests that this particular blend (i.e. 12.3% H_2O) represents approximately the situation where both these types of behaviour are about equally balanced, resulting in an almost constant value of ε/k ÷ 1000 over the whole temperature range examined. For the further elaboration of these ideas, it is necessary

(a) to extend the experiments to the region of lower as well as higher water-content so as to get a complete picture of these interesting phenomena, and

TABLE 3.—MEAN RESULTS OF MEASUREMENTS OF ε′/k ÷ 1000 ON ALCOHOL CONTAINING 12.3±0.5% WATER.

Temperature (°C.)	10.1	12.0	14.0	16.0	18.0	20.0	22.0	24.0	26.0	28.0	30.0	32.0
ε′/k ÷ 1000	2.080 ±.020	2.060 ±.010	2.080 ±.016	2.093 ±.019	2.055 ±.004	2.066 ±.007	2.059 ±.005	2.064 ±.016	2.054 ±.006	2.028 ±.012	2.046 ±.005	2.057 ±.009
Temperature (°C.)	34.0	36.0	38.0	40.0	42.0	44.0	46.0	48.0	50.0	52.0	54.0	—
ε′/k÷ 1000	2.058 ±.001	2.036 ±.009	2.028 ±.007	2.039 ±.015	2.025 ±.014	2.008 ±.013	2.015 ±.009	1.991 ±.002	2.032 ±.020	2.016 ±.020	1.963 ±.010	—

NOTE:—R. M. S. Standard Deviation for the whole temperature range = ± 0.012

(b) to improve the accuracy of the foregoing measurements by a factor of five to ten, if possible.

Experiments along these lines are in hand, and their results will be reported in later communications.

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CHEMICAL STUDIES IN RELATION TO THE BIOSYNTHESIS OF SOME NATURAL BENZOFURANS AND RELATED COMPOUNDS. PART I*

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6-Methoxycoumaranone (I) on reduction with sodium borohydride gives 6-methoxybenzofuran (IV). Condensation of acetone with (I) gives (III), also obtained from (II) by the action of alkali. On the basis of these reactions and of structural relations in natural furanocoumarins and furanoquinolines and related compounds, the hypothesis is put forward that the furan ring is biogenetically related to an *iso*prenoid side-chain.

Cyclisation of the epoxide of suberosin results in the formation of (XV). Methyl ester of coumarilic acid on reduction with lithium aluminium hydride gives (XX). Further reduction of (XX) with sodium in alcohol results in the formation of (XXI). The epoxides (X) and (XVII) on treatment with alcoholic alkali give (XVI) and (XVIII), respectively. On the basis of these reactions and of structural relationship the hypothesis is put forward that epoxidation of *iso*pentenyl and probably allyl side-chains is an essential phase in the process of development of furan ring fused to benzene nucleus and that coumarilic acid is involved in a different route in the biogenesis of these compounds.

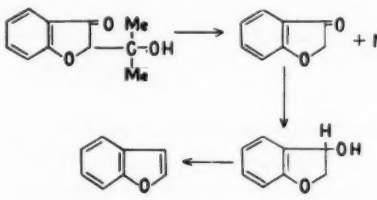
Introduction

The view has been advanced by Birch, Elliott and Penfold[1] that in the biosynthesis of certain resorcinol and phloroglucinol derivatives the *iso*pentenyl side-chain could be introduced *en bloc* in a stage independent of the synthesis of the nucleus. An examination of the structures of a number of natural coumarin derivatives and related compounds, based chiefly on the review by Geissman and Hinreiner[2] supports this idea. The *iso*pentenyl side-chain in some cases contains oxygen; in others it is cyclised to give an *iso*propylfuran derivative or a dimethyl chroman ring system and also there are a number of these compounds in which the side-chain is linked with oxygen rather than with carbon.

The present investigation was undertaken with a view to providing some chemical basis for a hypothesis to explain the formation of an unsubstituted furan ring fused to a benzene nucleus. Several suggestions can be made to explain the biosynthesis of the unsubstituted furan ring: (i) it may be derived from a C_2 side-chain such as acetyl; (ii) it may result by loss of an *iso*propyl group from an *iso*propylfuran derivative formed by cyclisation of an *iso*pentenyl side-chain; or (iii) it may be produced by cyclisation of an *o*-hydroxyphenylacetaldehyde formed by oxidation of an *iso*pentenyl or allyl side-chain.

There is no evidence for or against (i), although plausible routes could be suggested. Route (ii) or (iii) is favoured by considerable structural evidence although against (iii) is the fact that not a single corresponding lactone is known. Oxidation of an intermediate cyclic half-acetal might be expected to occur in a few cases at least. The unsubstituted furan derivatives and *iso*propylfuran derivatives occur commonly in the same plant genera, and *iso*prenoid side-chains are found attached to oxygen in both types of compounds. In the genus *Peucedanum* are found: peucedanin (2′-*iso*propyl-3′-methoxy-1); *iso*imperatorin (5 *iso*pentenyloxy-1); ostruthol (5-$OCH_2CH(OCOO\text{-}(CH_3) = CH.CH_3)$ CH $(CH_3)_2OH$-1);oxypeucedanin (5-epoxy-*iso*pentenyloxy-1); nodakenin (2′,3′-dihydro-2′-glucosoxy*iso*propyl-1); athamantin (2′, 3′-dihydro-2′ - hydroxy*iso*propyl-3′-hydroxydi-*iso*valerylester-11), together with the bicyclic coumarins umbelliferone (7-hydroxy-), ostruthin (7-hydroxy-6-geranyl-), and osthol (7-methoxy-8-*iso*pentenyl-).

The most probable mechanism for the formation of coumarones would consist in the removal of an *iso*propyl side-chain by means of a reverse-aldol reaction as shown below followed by reduction.



It is notable that in all but one of the cases containing an *iso*propylfuran ring, the group is either hyrdoxy*iso*propyl or *iso*propenyl, and in all the dimethylchroman derivatives and in many of those naturally occurring compounds which con tain *iso*pentenyl side-chains attached to oxygen the carbon bearing the gem-dimethyl group has an oxygen attached. Furthermore two out of three furanocoumarins retaining the *iso*propyl group have no oxygen in the 3′-position suggesting that

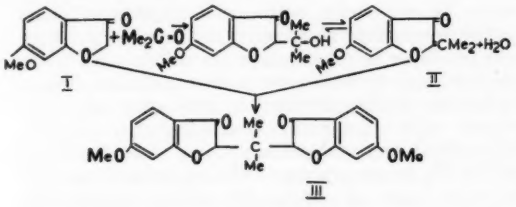
*This work was carried out in the Department of Organic Chemistry, University of Sydney, Sydney, Australia

these substances (peucedanin, athamantin, all in *peucedanum*) all represent arrested stages to unsubstituted furan derivatives.

Only biological work can ultimately prove or disprove such a hypothesis, but the reactions of some coumaranones have been investigated in relation to the above ideas.

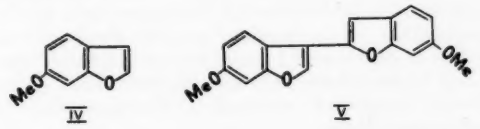
I. Reactions of 6-Methoxy Coumaranone.—In order to provide some basis for a hypothesis such as above the reactions of 6-methoxycoumaran-3-one (I)[3] were first examined.

The *iso*propylidene derivative (II)[4] has been obtained by the condensation of coumaranone (I) and acetone in presence of zinc chloride, and a reversal of such a condensation would provide the most likely route for the loss of an *iso*propyl side-chain. Compound (II) on heating with alkali, for preference in the presence of sodium hydrosulphite to reduce oxidative side-reactions, gave evidence of such reversal by the formation of (III) and acetone. The compound (III) is formed with great ease by reaction between (I) and (II) and is the only product isolated after treating the coumaranone (I) with alkali and a large excess of acetone. These results indicate the ready occurrence of the postulated reversed aldol formation, the process being that below:—

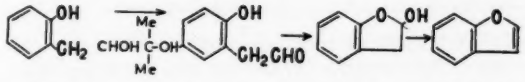


If this view of the biosynthetic process is correct, coumaranones such as (I) should be readily convertible to the furans by reduction. This was demonstrated by reducing (I) with sodium borohydride to obtain preponderantly (IV) with a small amount of a substance which appears to be a reduction product of an analogue of oxindirubin formed. Structure (V) is assigned to this compound on the basis of the known oxygen analogues of indirubin.[5,6] The formation of the compound (V) under these conditions shows how readily (I) undergoes self-condensation followed by reduction and dehydration. The 6-methoxy-coumarone, n_D^{18} 1.156647 gave the picrate,[7] m.p. 64°C. Reduction of (II) with a limited amount of sodium borohydride gave an oily monomeric product which appeared to consist mainly of (IV) together with (III).[4] Thus it appears that the borohydride reacts as a base as well as a reducing agent and the *iso*propylidenecoumaranone is sluggishly reduced.

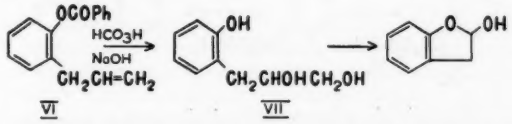
These experiments show that at least a feasible series of chemical mechanisms exists whereby the biosynthetic hypothesis (ii) outlined above could be effective.



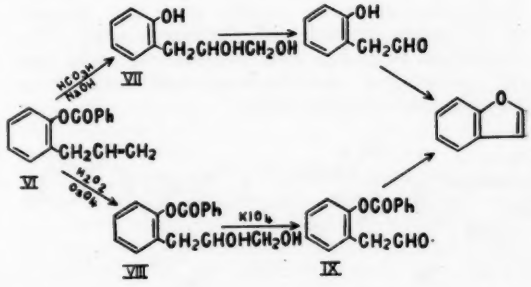
II. Cyclisation of 2-hydroxyphenylacetaldehydes.—The common presence of *iso*prenoid side-chains with the group oxidised to the glycol or the epoxide suggests that such groups might represent arrested stages to the phenylacetaldehyde which could cyclise with an *o*-hydroxyl group to the 2-hydroxydihydrofuran. Dehydration could then give the benzofuran:



To examine the chemical feasibility of this series of reaction the following series of reactions was examined:



The glycol (VII) was readily obtained, but oxidation with either lead tetraacetate or periodic acid produced only polymeric material, although formaldehyde could be detected. If this route were invovled in biosynthesis, the lactones corresponding to the intermediate acetal would be expected as natural products; in fact no such compound is known.

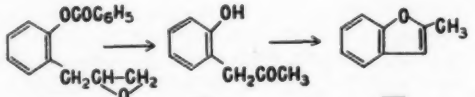


It, therefore, appeared necessary that the benzoyl group of (VI) should be retained for the subsequent steps of oxidation. The first step of oxidation which was accomplished by the action of hydrogen peroxide on *o*-benzoyloxy-allyl-

benzene in the presence of osmium tetroxide[8] resulted in the formation of 1-(*o*-benzoyloxyphenyl)-propan-2 : 3-diol (VIII). The next step was carried out by oxidising (VIII) with periodic acid. The product obtained was *o*-benzoyl-oxyphenylacetaldehyde (IX), characterised by the preparation of a semicarbazone. Attempts to hydrolyse the benzoyl group of the aldehyde with dilute hydrochloric acid or sulphuric acid under various conditions resulted in the formation of resinous products.

These results coupled with structural evidence lend support to the view that furan rings are not likely to arise in nature by the oxidation of glycollic C_5 or C_3 side-chains. The other possibility that furan rings might be formed in nature by the cyclisation of *iso*pentenyl or allyl side-chains containing an epoxide ring was then examined by a study of the reactions of the epoxides of suberosin and *o*-benzoyloxy-allylbenzene.

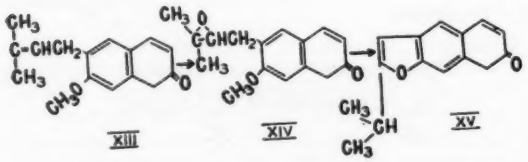
Treatment of such an epoxide with acid was expected to result in hydrolysis and rearrangement to a ketone followed by cyclisation to 2-methyl-coumarone.



X XI XII

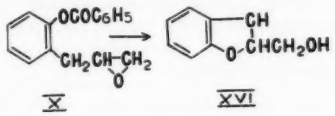
Accordingly *o*-benzoyloxyallylbenzene was converted to the epoxide (X) by treatment with perbenzoic acid, but there was no indication of the formation of postulated cyclisation product (XII) nor could the isomeric ketone (XI) be isolated after treatment of the epoxide with dilute sulphuric or phosphoric acid.

III. Cyclisation of the Epoxides of Isopentenyl and Allyl Side-chains.—Suberosin (XIII)[9] extracted from *Zanthoxylum suberosum* readily gave the epoxide (XIV) when treated with perbenzoic acid. The epoxide on refluxing with hydrobromic acid and red phosphorus demethylated and cyclised to anhydro-nodakenetin (XV).[10]



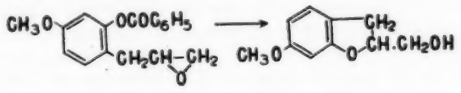
XIII XIV XV

Treatment of the epoxide of *o*-benzoyloxy-allylbenzene with alcoholic sodium hydroxide solution resulted in the formation of a substance $C_9H_{10}O_2$, which was insoluble in alkali and gave no colour with ferric chloride and no derivative with 2:4-dinitrophenyl-hydrazine hydrochloride. However, a derivative was obtained with 3:5-dinitrobenzoyl chloride, and a Kuhn-Roth determination of this derivative showed that there was no C-CH_3 group present. The properties of this cyclisation product suggest that it may be identical with 2-hydroxymethylcoumaran (XVI).[11]



X XVI

A similar cyclisation product was obtained from the alkaline treatment of the epoxide of 2-benzoyloxy-4-methoxy-allylbenzene (XVII).



XVII XVIII

The allyl ether of monomethyl-resorcinol was prepared by the method described by Claisen[12] and rearranged to 2-hydroxy-4-methoxy-allyl-benzene.[13] This compound gave a benzoate which readily gave the epoxide with perbenzoic acid. In alcoholic sodium hydroxide solution cyclisation occurred to give a compound which was insoluble in alkali, gave no colour with ferric chloride and no derivative with 2:4-dinitrophenyl-hydrazine hydrochloride. A derivative was obtained with 3:5-dinitrobenzoylchloride and a Kuhn-Roth determination showed that there was no C-CH_3 group present. The cyclisation product in this case is probably 2-hydroxymethyl-6-methoxy-coumaran (XVIII).

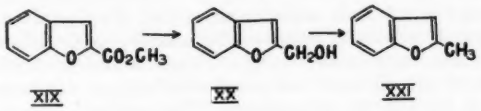
It seems clear, therefore, that the epoxides of isopentenyl side-chains can be converted to coumarones under acid conditions. Further work on the behaviour of the epoxides of *o*-hydroxy-allylbenzenes under mild alkaline conditions is necessary. It is obvious that cyclisation does occur in the latter cases and the products isolated suggest that this route may be involved in the biosynthesis.

IV. Decarboxylation and Reduction of Coumarilic Acid.—Apparently decarboxylation of coumarilic acid derived from coumarins constitutes another biologically possible mechanism but since decarboxylation does not occur readily[14] this route is less likely to be involved in the biosynthesis of unsubstituted furan rings.

2-Methylbenzofuran (XXI) was, however, obtained by reducing the methyl ester of coumarilic

acid in two stages. Reduction of (XIX) with lithium aluminium hydride gave the alcohol (XX), confirmed by the preparation of a derivative, m.p. 133°C., with 3:5-dinitrobenzoyl chloride.

Further reduction of the alcohol (XX) with sodium in alcohol resulted in the formation of (XXI).



XIX XX XXI

Experimental

Melting points are uncorrected. Microanalyses were carried out partly by the C.S.I.R.O. Microanalytical Laboratory and partly by the Microanalytical Laboratory, University of Sydney.

(*a*) *6-Methoxycoumaranone.*—This compound was obtained from α-bromo-resacetophenone-dimethyl-ether and potassium acetate by the method described by Blom and Tambor.[3]

(*b*) *Reduction of 6-Methoxy-coumaran-3-one with Sodium Borohydride.*—6-Methoxy-coumaran-3-one (0.5 g.) was dissolved in absolute ethanol (20 ml.). An excess (20%) of sodium borohydride dissolved in absolute ethanol was added to the warmed solution, and the mixture refluxed for one hour. After cooling, it was acidified with hydrochloric acid (5N), allowed to stand for two hours, diluted and extracted with ether and the solvent evaporated to small bulk, then passed through a column of alumina. The washings, on evaporation, gave a mixture of solid and oil which were separated by filtration.

The solid was recrystallised from ethanol, m.p. 144-5°C. (Found: C, 738.4; H, 4.9. Calc. for $C_{18}H_{14}O_4$: C, 73.3; H, 4.8).

The oil, b.p. 67°C./1.5-2.0 mm., n_D^{18} 1.5664, (Found: C, 72.9; H, 5.5. $C_9H_8O_2$ requires: C, 73.0; H, 5.4), gave a picrate,m.p. 64°C., and a trinitrobenzene derivative, m.p. 56°C.

(*c*) *Reduction of 6-Methoxy-2-isopropylidene-coumaran-3-one with Sodium Borohydride.*—6-Methoxy-2-*iso*propylidene-coumaran-3-one (1.5 g.) was treated with sodium borohydride as above. The solid obtained from the reaction mixture was removed by filtration and recrystallised from absolute ethanol, m.p. 203-4°C., not depressed in mixed melting-point with an authentic specimen of 2,2-bis-(6′-methoxy-coumaran-3′-one)-propane. (Found: C, 68.7, H, 5.5, $C_{21}H_{20}O_6$ requires: C, 68.5; H, 5.5).

After removal of the solid, the mother liquors were extracted with ether, which was dried over sodium sulphate and then removed by distillation under reduced pressure. The residual oil distilled at 80°C./0.8 mm. (Found: C, 73.3; H, 6.2. $C_9H_8O_2$ requires: C, 73.0; H, 5.4).

(*d*) *Self-condensation of 6-Methoxy-2-isopropylidene-coumaran-3-one.*—6-Methoxy-2-isopropylidene-coumaran-3-one (100 mg.) was dissolved in absolute ethanol (10 ml.) and a solution of sodium hydrosulphite (50 mg.) in sodium hydroxide solution (0.5 ml., 10%) added. The mixture was refluxed on a water bath under nitrogen for forty minutes and distilled into 2:4-dinitrophenylhydrazine hydrochloride solution. The yellow precipitate was identified as acetone 2:4-dinitrophenylhydrazone. After cooling, a solid separated from the residue and was recrystallised from absolute ethanol, m.p. 203-204°C., not depressed in mixed melting point with an authentic specimen of 2,2-bis-(6′-methoxy-coumaran-3′-one-)-propane.[4]

(*e*) *Condensation of 6-Methoxy-2-isopropylidene-coumaran-3-one with 6-Methoxy-coumaran-3-one.*—6-Methoxy-2-isopropylidene-coumaran-3-one (50 mg.) and 6-methoxy-coumaran-3-one (50 mg.) were dissolved in absolute ethanol. The system was filled with nitrogen and sodium hydroxide solution (2 drops, 10%) added. On standing, a solid separated and was recrystallised from absolute ethanol, m.p. 203-204°C., not depressed in mixed melting-point with an authentic specimen of 2,2-bis-(6′-methoxy-coumaran-3′-one)-propane.[4]

No acetone 2:4-dinitrophenylhydrazone could be obtained from the mother liquors.

(*f*) *Benzoylation of o-Allylphenol.*—*o*-Allylphenol[12] (67 g.) in pyridine (79 g.) was treated with excess freshly-distilled benzoyl chloride and the mixture was left overnight. After dilution with water it was extracted with ether and the extract washed with hydrochloric acid (5N; 4 × 25 ml.), saturated sodium bicarbonate solution, sodium hydroxide solution (5%) and water. After drying over anhydrous sodium sulphate the solvent was evaporated and the residue distilled, b.p. 162°C./1.5 mm. Approximately, the theoretical yield of benzoate was obtained. (Found: C, 80.5; H, 6.1. $C_{16}H_{14}O_2$ requires: C, 80.1; H, 5.9).

(*g*) *1-(o-Hydroxyphenyl)-propan-2:3-diol.*—The benzoate of *o*-allylphenol (11.9 g., 0.05 M) was dissolved in formic acid (42.3. ml.) at 25°C. Hydro-

gen peroxide (5.9 g., 30%, 0.0513 M, 2.5% in excess) was added gradually, the temperature being maintained at 40°C. After standing two hours at this temperature, the bulk of formic acid was removed by distillation under reduced pressure in an atmosphere of nitrogen. The mixture was diluted with water and extracted with ether and then ethyl acetate. The combined extracts were washed with saturated sodium bicarbonate solution and after drying the solvents were evaporated. The oily residue could not be induced to crystallise and was hydrolysed by refluxing with excess aqueous methanolic sodium hydroxide for two hours.

After cooling, the solution was saturated with carbon dioxide and the methanol removed by distillation. The slightly alkaline solution was extracted with ether. Evaporation of the solvent gave 1-(*o*-hydroxyphenyl)-propan-2:3-diol, b.p. 175°C./1.2 mm. (Found: C, 64.0; H, 7.3. $C_9H_{12}O_3$ requires: C, 64.3; H, 7.2).

(*h*) *Oxidation of the Benzoate of o-Allylphenol with Hydrogen Peroxide in the Presence of Osmium Tetroxide.*—A three-necked flask was fitted with a mechanical stirrer, two dropping funnels and a reflux condenser. To a mixture of anhydrous sodium sulphate (10 g.), absolute ether (20 ml.) and ethereal osmium tetroxide (2 ml., 1%), were added dropwise, ethereal solutions of hydrogen peroxide (7.3 N) and the benzoate of *o*-allylphenol (2.5M, 11.9 g.). The addition of the benzoate (1 ml.) resulted in the formation of a brown complex with osmium tetroxide. Hydrogen peroxide was then added alternately with the benzoate and the mixture was stirred until it became light yellow. Next day carbon dioxide was passed through the flask with stirring to remove any formaldehyde. The mixture was diluted, the ether layer separated and washed several times with water, sodium hydroxide (1%), sodium bisulphite and water. After drying over sodium sulphate the solvent was evaporated when the residue crystallised. 1-(*o*-Benzoyloxy-phenyl)-propan-2:3-diol, m.p. 82°C., was recrystallised from benzene as white plates. (Found: C, 70.6, H, 5.8. Calc. for $C_{16}H_{16}O_4$: C, 70.6; H, 5.9).

(*i*) *Oxidation of 1-(o-Benzoyloxyphenyl)-propan-2:3 diol with Periodic Acid.*—1-(*o*-Benzoyloxyphenyl)-propan-2:3-diol (2 g.) was dissolved in ethanol (100 ml., 70%). Potassium (meta) periodate (200 ml., 5.7 g. in N H_2SO_4) was rapidly added. After two and a half hours the solution was extracted with ether, the extract washed with saturated sodium bicarbonate solution, and evaporated.

Treatment of a small portion of the residue with semicarbazide hydrochloride gave *o*-benzoyloxyphenyl-acetaldehyde semicarbazone, m.p. 184°C., which crystallised from absolute ethanol (Found: C, 64.4; H, 5.1. Calc. for $C_{16}H_{15}O_3N_3$: C, 64.6; H, 5.1).

(*j*) *Epoxidation of o-Benzoyloxy-allylbenzene.*—*o*-Benzoyloxy-allylbenzene (11.9 g., 0.05 M) was added to a solution of perbenzoic acid in chloroform (140 ml., 7.6 g., 10% in excess of 0.05 M) and the solution maintained at 0°C. for four days. Benzoic acid was removed by washing with dilute sodium hydroxide (5%). After washing with water the solution was dried over sodium sulphate and the solvent evaporated. The residual oil was distilled, the fraction boiling at 173°C./1.5 mm. being collected. This fraction consisted of the epoxide of *o*-benzoyloxy-allylbenzene. (Found: C, 75.9; H, 5.7. Calc. for $C_{16}H_{14}O_3$: C, 75.6; H, 5.6).

(*k*) *Alkaline Hydrolysis of the Epoxide of o-Benzoyloxy-allylbenzene.*—The epoxide of *o*-benzoyloxy-allylbenzene (20 g.) was dissolved in absolute ethanol (50 ml.) and sodium hydroxide (50 ml.; 20%) added. The mixture was allowed to stand in a stoppered flask at room temperature for five days. The alkaline solution was diluted with water and the separated oil extracted with ether. The solvent was washed several times with water and dried over sodium sulphate. After evaporation of the solvent 2-hydroxymethyl-coumaran was distilled, b.p. 108°C./1.0 mm. (Found: C, 71.7; H, 6.3. $C_9H_{10}O_2$ requires: C, 72.0; H, 6.7). The yield was 60% of the theoretical amount.

(*l*) *Preparation of 3:5-Dinitrobenzoate of 2-Hydroxymethyl-coumaran.*—3:5-Dinitrobenzoyl chloride (1 g.) was dissolved in benzene (10 ml.) and 2-hydroxymethyl-coumaran (1 g.) dissolved in pyridine (2 ml.) added. The mixture was refluxed for thirty minutes. After cooling it was diluted with ether and washed with hydrochloric acid (2N), sodium hydroxide (5%) and water. After drying over sodium sulphate the solvents were evaporated and the solid derivative, m.p. 114.5°C. recrystallised from light petroleum, b. p. 60-90°C. (Found: C, 55.9; H, 3.6. Calc. for $C_{16}H_{12}O_7N_2$: C, 55.8; H, 3.5). A Kuhn-Roth determination was negative.

(*m*) *Benzoylation of 2-Hydroxy-4-methoxy-allylbenzene.*—2-Hydroxy-4-methoxy-allylbenzene (16.4 g., 0.1 M) was benzoylated as in (f). The reaction mixture, after dilution with water, was extracted with ether and the extract washed with hydrochloric acid (5 N, 4×25 ml.), sodium hydroxide (10%) and water. After drying over sodium sulphate the solvent was evaporated and the residue distilled. The benzoate distilled at 173°C./

1.1 mm. (Found: C, 76.4; H, 5.6. Calc. for $C_{17}H_{16}O_3$: C, 76.1; H, 6.0).

(*n*) *Epoxidation of 2-Benzoyloxy-4-methoxy-allylbenzene.*—2-Benzoyloxy-4-methoxy-allylbenzene (13.4 g., 0.05 M) was added to a solution of perbenzoic acid in chloroform (140 ml., 7.59 g., 10% in excess of 0.05 M) and the solution maintained at 0°C. for forty-eight hours. Benzoic acid was removed by washing with dilute sodium hydroxide (5%). After washing with water the solution was dried over sodium sulphate and the solvent evaporated. The epoxide of 2-benzoyloxy-4-methoxy-allylbenzene distilled at 196°C./0.75 mm. (Found: C, 72.6; H, 5.7. Calc. for $C_{17}H_{16}O_4$: C, 72.8; H, 5.7).

(*o*) *Alkaline hydrolysis of the Epoxide of 2-Benzoyloxy-4-methoxy-allylbenzene.*—The epoxide of 2-benzoyloxy-4-methoxy-allylbenzene (5 g.) was dissolved in absolute ethanol (25 ml.) and sodium hydroxide (25 ml. 20%) added. The mixture was kept at room temperature for five days. The alkaline solution was diluted with water and extracted with ether. The extract was washed several times with water and dried over sodium sulphate. After evaporation of the solvent the residual oil was distilled, b.p. 138°C./1.5 mm. It was insoluble in dilute alkali and gave no colour with ferric chloride. Treatment with 3:5-dinitrobenzoyl chloride gave a derivative, m.p. 140°C. (Found: C, 54.6; H, 3.8. Calc. for $C_{17}H_{14}O_7N_2$: C, 54.6; H, 3.8). A Kuhn-Roth determination was negative.

(*p*) *Epoxidation of Suberosin.*—Suberosin (12.2 g., 0.05 M) was added to perbenzoic acid in chloroform (150 ml., 7.6 .g, 10% in excess of 0.05 M). The mixture was maintained at 0°C. for twenty-four hours. Benzoic acid was removed by washing with sodium hydroxide (10%) and the chloroform washed with water and dried over sodium sulphate. After evaporation of the solvent the epoxide of suberosin was recrystallised from methanol, m.p. 120-1°C. (Found: C, 69.1; H, 6.2. Calc. for $C_{15}H_{16}O_4$: C, 69.2; H, 6.2).

(*q*) *Demethylation and Cyclisation of the Epoxide of Suberosin.*—The epoxide of suberosin (2 g.) and red phosphorus (1 g.) were added to hydrobromic acid (150 ml., sp. gr. 1.5). The mixture was refluxed for one hour, diluted with water and extracted with ether (3 × 30 ml.). The extract was washed with sodium hydroxide (0.5%), then with water, and the solvent evaporated. Anhydro-nodakenetin, m.p. 136°-7°C., was recrystallised from methanol. (Found: C, 73.5; H, 5.3. $C_{14}H_{12}O_3$ requires: C, 73.7; H, 5.3).

(*r*) *Methyl Coumarilate.*—A round-bottomed flask (250 ml.) containing absolute methanol (50 ml.) was cooled in an ice bath and dry hydrogen chloride was passed until the increase in weight was two grammes. Coumarilic acid (10 g.) was added and the mixture refluxed for four hours. After cooling it was diluted with water and extracted with ether. The ethereal solution was washed with saturated sodium carbonate solution and then with water, and dried over anhydrous potassium carbonate. After evaporation of the solvent, the ester was recrystallised from ether, m.p. 54-5°C.

(*s*) *2-Hydroxymethyl-coumarone.*—Lithium aluminium hydride (1.08 g., twice the required amount) was rapidly transferred to a three-necked flask (300 ml.) provided with a mechanical stirrer, dropping funnel and a reflux condenser. Dry ether (25 ml.) was added immediately. The mixture was stirred continuously for two hours after the addition had been completed. The flask, was then placed in an ice bath and water added dropwise cautiously to decompose the excess hydride. The ether solution was separated by filtration and dried over anhydrous sodium sulphate. After evaporation of the solvent, the oil distilled, b.p. 108°C./1 mm. Treatment of a small portion of this oil with 3:5-dinitrobenzoyl chloride gave a derivative, m.p. 133°C., from benzene/ether. (Found: C, 56.3; H, 3.1. Calc. for $C_{16}H_{10}O_7N_2$: C, 56.1; H, 3.0).

(*t*) *2-Methyl-coumarone.*—Sodium (2.48 g.) was placed in a two-necked round-bottomed flask (250 ml.) provided with a reflux condenser and dropping funnel. 2-Hydroxymethyl-coumarone (1.0 g.) dissolved in dry ethanol (25 ml.) was added in several portions. A vigorous reaction took place and, after it had subsided, the mixture was refluxed on a water-bath for one hour. After cooling, water was added and the mixture extracted with ether. The ethereal extract was washed with water and dried over sodium sulphate. The solvent was evaporated and the oil distilled, b.p. 52°C./2-0 mm. (Found: C, 81.8; H, 6.0. C_9H_8O requires: C, 81.8, H, 6.1).

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STUDIES ON CENTAUREA BEHEN LINN. (COMPOSITAE). PART II

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The alcohol, water and piperidine extracts of *Centaurea behen* Linn. have been investigated. A glucoside, m.p. (dec.) 210-285°C., and $[\alpha]_D^{30} = -48°$ (0.5% solution in tetrahydrofuran), has been isolated from the alcohol extract. On hydrolysis the glucoside yielded glucose and the new centaurea sterol A (C_{27-28} H_{44-48} O), m.p. 133-134°C., $[\alpha]_D^{25} = -31°$. The alkaloid was present in the plant in a very small amount and did not yield sufficient material to be characterised. The reducing sugars were obtained in 23.4% yield from the aqueous extract. From the residual roots, inulin was obtained up to 38% of the fresh roots.

The alleged therapeutic importance of Centaurea behen roots in jaundice, diabetes and calculus affections cannot be attributed to the compounds isolated from the petroleum ether extract and discussed in Part I.[1] On the other hand the earlier workers, as mentioned in Part I, also failed to isolate any well-defined compound to which the therapeutic properties of Centaurea behen roots can be assigned. In this second part, the exhaustively petroleum-ether-extracted roots have been extracted with alcohol, alcohol containing 2% hydrochloric acid, cold water and finally with piperidine, and the products obtained may perhaps throw some light on the pharmacological efficaciousness of the plant.

The alcoholic extract furnished a large amount of a dark brown honey-like residue. The first two extractions on concentrating deposited a colourless amorphous globule-shaped substance which was purified by washing with petroleum ether and hot alcohol. Recrystallisation three times from tetrahydrofuran led to the pure globule shaped substance showing a constant rotation of $[\alpha]_D^{30} = -48°$ in 0.5% tetrahydrofuran solution. Found: C, 72.1; H, 10.38; O, 17.27 which fits in the range of C_{33-35} $H_{54-60}O_6$. It did not melt but decomposed slowly between 210-285°C. It was soluble in hot tetrahydrofuran, hot dimethyl formamide, pyridine, piperidine, very sparingly so in alcohol and acetone, and insoluble in petroleum ether, benzene, chloroform, ethyl acetate, carbon tetrachloride and water. With concentrated sulphuric acid it gave red colouration which intensified on warming but on further heating on a water bath the substance charred. After hydrolysis with 2N hydrochloric acid it gave needle shaped crystals and the solution reduced Fehling's solution which indicates a glucoside.

Hydrolysis of the glucoside with hydrochloric acid gas in alcohol led to a colourless sterol, m.p. 133-4°C., which crystallized in the form of leaflets from alcohol or acetone and in the form of needles from petroleum ether or ethyl acetate. The specific rotation was constant at $[\alpha]_D^{25} = -31°$ in 1% chloroform solution. The analysis C, 83.61; H, 11.8; O, 4.41 would agree best with the formula C_{27-28} $H_{44-48}O$. The difference between the formula of the glucoside and that of its aglucone indicates the sugar part to be a hexose which was proved to be glucose by paper chromatographic analysis as described in the experimental part. The infrared spectrogram of the sterol is given in Fig. 1. The positive Lieberman Burchard test, digitonin test and decolourisation of potassium permanganate in alkaline solution and bromine water indicated a sterol having a hydroxyl group in 3β-position and a double bond. Comparison of centaurea sterol and its acetate with other similarly melting sterols and their acetates, known up to 1957 is given in Table 1.

The alcoholic extract gave positive alkaloidal tests, but not in encouraging amounts. Efforts were made to isolate the little amount of the alkaloid present by solvent extraction and by precipitation with Dragendorff's reagent. The decomposition of the latter with hydrogen sulphide or with silver oxide was not successful.

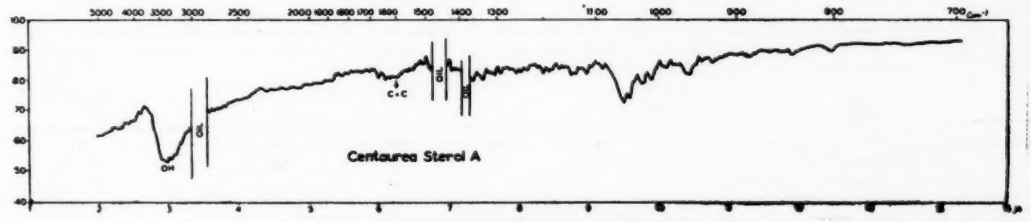

Fig. 1.—Infrared spectrogram of centaurea sterol A.

Subsequently the roots were again extracted with alcohol but 2% hydrochloric acid was added to get actually a higher percentage of alkaloid. Again large quantities of a substance came out in the blackish extract which on heating during concentration deposited huge amounts of charcoal which actually filled the distillation flasks completely. Only sugars could behave like that. Therefore, the alcohol-extracted roots in new experiments were exhaustively extracted with cold water yielding 21% of brownish residue by weight of the petroleum ether and alcohol-extracted roots. It contained 24% reducing sugars in organic salts but no alkaloid. This amount of sugar, however, could not account for the large amount of sugar coal received with alcohol containing 2% hydrochloric acid. However, the glucoside being insoluble in alcohol and water could still be expected to be present in the roots. Therefore, they were subsequently soxhleted with tetrahydrofuran, a solvent for the glucoside. But only a little amount of oily substance was obtained, from which no glucoside could be isolated. By a test extraction with cold piperidine, however, a globule-shaped white substance could be observed in fairly large amounts specially on adding alcohol to the piperidine extract. Thus 54.3% by weight of the roots extracted with petroleum ether, alcohol and water and dried afterwards were obtained which equal to 37.8% by weight of the fersh roots. This substance was quite different from centaurea glucoside. 2N Sodium hydroxide and hot 2N hydrochloric acid dissolve it easily due to hydrolysis. Ten times dissolved in hot water and precipitated with an excess of alcohol. A preparation of $[\alpha]_D^{26} = -34°$ in 1% aqueous solution was obtained. Shrinking and softening starts at 160-195°C., decomposition between 195-210°C.,

TABLE 1.—COMPARISON OF CENTAUREA STEROL A AND ITS ACETATE WITH SIMILAR STEROLS AND THEIR RESPECTIVE ACETATES KNOWN UP TO 1957.

Sterol	Centaurea sterol A	Sterol form Beach Bark[2]	Slanu-tosterol[3]*	Yasudes-terol[4]	Raphanis-terol[5]*	Verosterol[6]*	Matsusterol[7]	Pectsterol[8]	β-Sitosterol[9]
Formula	$C_{27-28}H_{44-48}O$	$C_{24}H_{40}O$	$C_{26}H_{42}O$	$C_{27}H_{46}O$	$C_{27}H_{46}O$	$C_{27}H_{46}O$	$C_{27}H_{46}O$	$C_{27}H_{46}O$ or $C_{29}H_{50}O$	$C_{29}H_{50}O$
Crystalline shape	Leaflets (alcohol or acetone) or needles (pet. ether or ethyl acetate)	Leaflet or needles	—	Scaly (methanol)	Needles (alcohol)	Leaflets	Needles (methanol)	—	Needles or leaflets
M. p.°C	133-34	134	136-7	134-5	136	135-6	130-1	134-7	137—7.5
Rotation	$[\alpha]_D^{25} = -31°$ in 1% chloroform solution	$[\alpha]_D^{18} = -31.3°$ (cholroform)	—	—	$[\alpha]_D^{20} = -32.2°$ (chloroform)	$[\alpha]_D^{15} = -33.6°$ (chloroform)	$[\alpha]_D^{15} = -33.5°$ (chloroform)	$[\alpha]_D^{28} = -34.7°$ (chloroform)	$[\alpha]_D^{24} = -36.7°$ (chloroform)
Lieberman Burchard test	Positive. Violet-Blue-Green	Positive. Violet-Dark Green	Positive	Positive (Blue colour)	Positive	—	Positive		Positive
Digitonin test	Positive	Positive	—	Positive	—	—	—	—	Positive
Double bond reactions	Positive	—	Positive	—	Positive	—	—	—	Positive
Crystalline shape of the acetate	Leaflets or needles	—	—	—	Needles (alcohol)	Needles (Acetic anhydride)	—	—	Elongated rectangular plates
M. p. of acetate°C	122-3	121-2	128	123-123.5	125	122-4	117	136.5-38	Partly at 122-3 and completely at 127
$[\alpha]_D$ of the acetate	$[\alpha]_D^{27} = -36°$ (1 % in chloroform)	$[\alpha]_D^{18} = -32.4$ (chloroform)	—	—	—	—	$[\alpha]_D^{15} = -20.9°$	—	—

*These three sterols are probably impure β-sito sterols, according to A. George.[10]

and complete charring at 210-212°C. It was proved to be a polysaccharide by charring with concentrated sulphuric acid, spot tests according to Feigl and positive Fehling's solution test after boiling with acid. The assumption to be inulin could be confirmed by paper chromatographic analysis of the hydrolysate of the sample in comparison with the hydrolysate of B. D. H. inulin and pure glucose. Furthermore the acetyl derivatives of the sample and B.D.H. inulin were prepared by Haworth and Streight's method.[11] In order to get the sample and its acetyl derivative of constant optical rotation, it was necessary to repeat the purification several times as it is the case with other poly-fructosans and their derivatives.[12] Comparison of centaurea inulin with B. D. H. inulin and the inulin with constant optical rotation according to the literature and their respective acetyl derivatives is given in Table 2.

Inulin is of commercial importance. It is used as a starting material for levulose and an ingredient of diabetic breads and food preparations due to the fact that it consists mainly of fructose being easily digestible by the diabetic patient. Inulin nitrate is used as an ingredient of detonator composition, as a sensitizer in dynamites and as an ingredient of lacquers.[13] Centaurea behen roots may therefore, become a useful source of inulin. Not knowing when the sample used in the present investigation was harvested and because the inulin content of plants varies considerably having its maximum in autumn and a minimum in spring time, the resulting percentage of 37.8% on the undried fresh roots might not represent the highest available amount. There are several other plants which contain high percentages of inulin, e.g., pyrethrum contains 57.7% at its highest; Inula 44% in autumn and 19% in spring; Arnica roots 9-7%; Helianthus tubers, Macrophyllus and Arctium lappa 20-50%; Taraxacum in October 24% and in March 1.74%; and Dahlia tubers 9.7 - 13.4%.[14] Of these, pyrethrum seems to

TABLE 2.—COMPARISON OF CENTAUREA INULIN AND ITS ACETYL DERIVATIVE WITH B. D. H. INULIN, PURE INULIN ACCORDING TO THE LITERATURE AND THEIR ACETYL DERIVATIVE.

Properties	Physical appearance	Method of isolation	Purification	Solubilities	M. p.	Sp. Rotation	Products of hydrolysis
Centaurea inulin (purified 10 times)	White amorphous powder	Extraction with piperidine or hot water and precipitation with excess of alcohol.	Dissolving in hot water and precipitation with alcohol	Similar	Shrinking, softening and foaming at 160-195°C. slow decomposition 195-210°C. complete dec. with charring 210-212°C.	$[\alpha]^{26}_{D} = -34°$ (1% aqueous solution)	Fructose and a very small amount of glucose.
B.D.H. inulin	,,	,,	,,	,,	Shrinking, softening and foaming at 155-195°C. slow decomposition 195-205°C. Complete dec. and charring 205-7°C.	$[\alpha]^{30}_{D} = -30°$ (1% aq. solution)	,,
Inulin according to the literature	,,	,,	,,	,,	—	$[\alpha]^{20}_{D} = -39°$ (in water)	Fructose and glucose. (12:1)
Acetyl derivative of c. inulin (purified 10 times)	White amorphous	Haworth or streight's method	Dissolving in chloroform or benzene & precipitating with pet. ether	,,	Shrinking, swelling and foaming at 65-115°C. Viscous at 115°C. melts at 140-50°C. to a colourless clear melt	$[\alpha]^{24}_{D} = -41°$ (1% chloro-solution)	—
Acetyl derivative of B. D.H. inulin (purified twice only)	,,	,,	,,	,,	Shrinking, swelling and foaming at 60-100°C., starts becoming viscous at 100°C. and melts at 120-30°C.	$[\alpha]^{24}_{D} = -30°$ (1% chloroform solution)	—
Inulin acetate according to literature	,,	,,	,,	,,	—	$[\alpha]^{20}_{D} = -34°$ (1.5% chloroform solution)	—

have the highest percentage of inulin but it cannot be made available because to win pyrethrum for insecticidal purposes only the flowers of this perennial plant are used continuously.[15] Actually inulin is prepared from Dahlia tubers by extraction with hot water.

Experimental

1. Extraction with Alcohol.—Centaurea behen roots, which had been exhaustively extracted with petroleum ether, were extracted with alcohol twelve times in the first experiment and seventeen times in the second experiment.

Experiment 1: 1.6 kg. of the roots, on extraction with 15 litres of distilled alcohol, yielded 105 g. of solvent-free extract (6.56% of the fresh roots).

Experiment 2: 7 kg. of the roots, on extraction with 76.5 litres of distilled alcohol, yielded 550 g. of solvent free extract (7.86% of the fresh roots).

2. Isolation of the Centaurea Glucoside.—11.5 litres of the first and second extracts of the second experiment, concentrated to about 700 ml., were kept overnight. A colourless amorphous substance settled down besides a little quantity of an oil. The amorphous substance was filtered under suction, washed with alcohol and dried on a porous plate. Yield of the crude product was 3 g. Some of the oily impurities were removed by shaking twice with petroleum ether and filtering. More of the impurities were subsequently removed by repeatedly heating with alcohol, cooling and filtering. The yield dropped down to 2 g. (0.03% of the fresh roots). This pretreated material was finally purified by recrystallising thrice from tetrahydrofuran wherefrom it crystallises in the form of globules. They were soluble in piperidine, pyridine, hot dimethyl formamide, hot dioxane, tetrahydrofuran, very sparingly so in alcohol and acetone and insoluble in petroleum ether, chloroform, carbon tetrachloride, ethyl acetate and water. The glucoside did not have any m.p. but decomposed slowly. At 210°C. it became light brown, at 235°C. brown, at 246°C. dark brown and finally it charred between 260-285°C. On treatment with concentrated sulphuric acid it became red. The colour intensified on warming and then the substance charred on prolonged heating on a water bath. With Liebermann-Burchard solution it gave blue colour which changed to green after a few minutes. It did not reduce Fehling's solution but, after heating with a few drops of 2N hydrochloric acid, there was at once reduction. The rotation ni 0.5% tetrahydrofuran solution was constant at $[\alpha]_D^{30} = -48°$. Found: C, 72.71; H, 10.38; O, 17.27 which fits into the range of C_{33-35}–H_{54-60} O_6.

3. Hydrolysis of the Glucoside to Centaurea Sterol A and Glucose.—400 mg. of the pure glucoside was refluxed with 40 ml. of alcohol on a water bath and dry hydrochloric acid gas passed through for about 20 minutes until the whole of the glucoside went in solution. Then the boiling was continued for about 15 minutes without passing hydrochloric acid gas. On cooling, the colourless needles of the aglucone separated. The whole was brought to dryness at about 50°C. under vacuum and the last traces of hydrochloric acid removed over potassium hydroxide in a vacuum desiccator. On adding water and petroleum ether to the residue, the aglucone went into the ether layer while the glucose was dissolved in water. The two layers were separated. The petroleum ether layer was washed with water, the solvent removed and the resulting aglucone crystals dried under vacuum. Yield, 268 mg.; m.p., 125-28°C. The crude aglucone was passed through a column 1 cm. × 15 cm. containing 20 g. of alumina (Brockman). Petroleum ether and ethyl acetate were used as eluants successively. With petroleum ether, only the small amount of impurities were removed. The aglucone which came out with ethyl acetate was collected in three fractions of 4 ml. each. First fraction melted at 127-29°C., second at 128-30°C., and the third one at 130-32°C. The first two fractions were recrystallised from alcohol. Thus obtaining the purity of the third fraction m.p. 130-32°C. mixed with fraction 3 and recrystallised twice more from alcohol, the m.p. was constant at 133-34°C. It was soluble in petroleum ether, ethyl acetate, acetone, tetrahydrofuran, chloroform, carbon tetrachloride, benzene, dioxane, sparingly so in alcohol, dimethyl formamide and insoluble in water. The aglucone crystallised in the form of needles from petroleum ether or ethyl acetate and in the form of leaflets from alcohol or acetone. With Liebermann Burchard solution the product gave a violet colour which changed through blue into green within a few seconds. Potassium permanganate in alkaline solution and bromine solutions were decolourised. Positive digitonin test indicated the presence of a hydroxyl group in 3β-positon. The specific rotation of 1% solution in chloroform was constant at $[\alpha]_D^{25} = -31°$. Found: C, 83.61; H, 11.8; O, 4.41 which agrees best with the formula C_{27-28} $H_{44-48}O$. The infrared spectrogram is shown in Fig. 1. For comparison with similar sterols, see Table 1.

4. Acetylation of the Centaurea Sterol A.—100 mg. of the sterol was refluxed with 3 ml. of acetic anhydride and two drops of pyridine for about

six hours. The solvent was removed at 50°C. under vacuum and the product dried in a vacuum desiccator over phosphorous pentoxide. Yield, 112 mg.; m.p., 117-20°C. The crystalline aglucone acetate was easily soluble in petroleum ether, ethyl acetate, chloroform, benzene, cyclohexane, butanol, dioxane, carbon tetrachloride, sparingly so in alcohol, acetone, dimethyl formamide and insoluble in water. The crystals were leaflets from acetone or alcohol and needles from ethyl acetate or methyl alcohol. After four recrystallisation with ethyl alcohol, the m.p. was constant at 122-23°C. The rotation of a 1% solution in chloroform was also constant at $[\alpha]_D^{27} = -36°$.

The water layer, after the hydrolysis of glucoside, reduced Fehling's solution. It was concentrated at 50-60°C. under vacuum to syrupy consistency. Yield, 140 m.g.

5. Identification of Glucose by Paper Chromatographic Analysis.—Whatman filter paper No. 1 of 18-inch length and 6-inch width was divided into two equal parts length-wise and a line perpendicular to these parts was drawn leaving four-inch space on one side of the paper. In the middle of one of these parts and on the perpendicular line 5 drops of an approximately 0.5% solution of the sample were placed with the help of a fine capillary tube in such a way that the next drop was placed when the foregoing one had dried up. Similarly 5 drops of approximately 0.5% solution of pure glucose were placed in the middle of the second part of the paper on the perpendicular line. The drops were not allowed to spread into a circle of more than 5 mm. diameter. Touching the paper with hand was also avoided. The paper was then hanged into the chromatographic chamber with its upper part in the boat. The perpendicular line was in the horizontal position.

The solvent was prepared from butanol, acetic acid and water which were taken in the ratio of 4:1:5, respectively, in a separating funnel and shaken thoroughly several times for about 15 minutes. After separation into two different layers, the lower water layer was poured into the trough of the chamber and the upper organic solvent layer was dropped into the boat with the help of a dropping funnel. The chamber was kept air tight. After 48 hours the paper was taken out, dried in air and sprayed first with approximately 0.5% aqueous silver nitrate solution in acetone and then with 2-3% aqueous sodium hydroxide solution in alcohol. After drying the spots were made permanent by washing the paper with dilute ammonia. The experiment was repeated once, but in this case the paper was kept in the chamber for 96 hours. In both these experiments the distance travelled by the sample spot was exactly equal to that of the pure glucose. This shows that the sample had the same RF value as glucose.

6. Attempt to Isolate the Alkaloid. Precipitation with Dragendorff's Reagent.—300 g. of the sovlent-free extract was dissolved in a minimum volume of 2N acetic acid and filtered from a small amount of insoluble material. To the filtrate Dragendorff's reagent was added until the precipitation was complete. The orange precipitate was filtered, washed with distilled water and dried in a vacuum desiccator over phosphorous pentoxide. The complex precipitate was 9 g. (3% of the extract and 0.21% of the fresh roots).

Method 1: 2 g. of the complex was treated with acetone containing a few ml. of 2N acetic acid. Some of the precipitate was insoluble. Hydrogen sulphide was passed through this suspension as long as there was some precipitation, and then filtered. The excess of hydrogen sulphide was removed with the solvent at 60°C., and the acidic residue alkalified with ammonium hydroxide was brought to dryness at 60°C. under vacuum. The residue was 1.956 g. Excess of 2N sodium hydroxide was added to the residue and extracted exhaustively first with ether and then with chloroform. Both ether and chloroform extracts were dried over anhydrous sodium sulphate, filtered and the solvents removed. The ether extract yielded 46 mg. of an oily material which gave no alkaloidal tests. The chloroform extract yielded 18 mg. only and gave positive alkaloidal tests. It was reddish brown and viscous mixed with some crystalline substance in it. It was sparingly soluble in petroleum ether, acetone, ethyl acetate, alcohol, chloroform, benzene and insoluble in water. From acetone needle-shaped crystals were obtained on the slide but the amount was too small for further processing. The extract was bitter in taste.

Method 2: To 1 g. of the complex suspended in 80 ml. of moist acetone, 1.5 g. of freshly prepared and dried silver oxide together with about 20 glass beads were added. The flask was shaken for about five hours. It was filtered and washed with acetone until nothing came out any more. After removing the solvent 94 mg. of an oily extract was obtained which gave positive alkaloidal tests but no crystals with any solvent or acid could be observed. The residue was then extracted exhaustively with chloroform. The solvent removed when only 8 mg. of a needle shaped crystalline substance remained which gave positive alkaloidal tests. Finally the residue was extracted with alchohol which yielded 49 mg. of a material

free from alkaloid.

This experiment was repeated with 4 g. silver oxide. After exhaustive extraction with acetone 134 mg. of residue was obtained. It gave positive alkaloidal test but no crystals with any solvent or acid. Subsequent extraction with chloroform did not yield any crystalline substance.

7. *Extraction with Water.*—Centaurea behen roots, which had been extracted exhaustively with petroleum ether and alcohol, were dried in air in the shadow for about 2 hours. 100 g. of these roots had to be extracted six times with 250 ml. of distilled water each time at room temperature until no substance went in solution any more. On bringing to dryness in vacuum at 60-70°C., 21 g. of a brown extract having a smell of burnt sugar was obtained corresponding to 21% of the petroleum ether and alcohol extracted roots.

The sweet tasting extract was easily soluble in water, sparingly so in dimethyl formamide, acetic anhydride and insoluble in methanol, ethyl acetate, petroleum ether, chloroform, tetrahydrofuran and piperidine. It did not contain any alkaloid but 23.4% reducing sugars, determined quantitatively by Fehling's solution method. It also contained inorganic salts.

8. *Extraction of Inulin with Piperidine.*—The roots, which had been exhaustively extracted with petroleum ether, alcohol and water, were dried completely. 60 g. of the dried roots were kept standing with 150 ml. of piperidine for two days at room temperature. The clear yellowish extract was filtered under suction and the extraction repeated 6 times. The combined extracts were precipitated with alcohol, the inulin separated as a white powder. It was filtered under suction, washed with alcohol in order to free it from piperidine and dried under vacuum. Yield, 32.572 g. = 54.3% of the roots extracted with petroleum ether, alcohol and water, which equals to 37.8% of the fresh roots with about 11% moisture. The inulin can be further purified by dissolving in hot water and precipitating with excess of alcohol. This process was repeated ten times when a preparation having $[\alpha]_D^{26} = -34°$ in 1% aqueous solution was obtained. Shrinking and softening starts at 160-195°C. Decomposition with colour change starts at 195-210°C. and complete charring at 210-212°C. It was easily soluble in hot water, hot pyridine, warm piperidine, hot dimethyl formamide, sparingly so in cold pyridine, cold dimethyl formamide and cold piperidine, nearly insoluble in cold water and insoluble in petroleum ether, tetrahydrofuran, benzene, chloroform, ethyl acetate, carbon tetrachloride, cyclohexane, dioxane, butanol and alcohol. 2N sodium hydroxide and hot 2N hydrochloric acid dissolve also easily due to hydrolysis. The confirmation of being a polysaccharide was done by positive Fehling's solution test after hydrolysis and spot tests according to Fig. 1. The Fehling's solution test was also slightly positive with a little reduction on boiling even before hydrolysis.

9. *Hydrolysis of the Centaurea Inulin and Paper Chromatographic Analysis of the Hydrolysates.*—To 1.g each of B.D.H. inulin and the sample from centaurea behen, 15 ml. of N sulphuric acid were added and allowed to stand for about six hours at room temperature (31°C.). During this time nearly all of both the samples had gone in solution. The sulphuric acid was precipitated completely as barium sulphate by drop-wise addition of barium hydroxide solution. After heating up to boiling it was cooled and filtered. Both the filtrates were diluted to about 0.5% concentration and compared with a 0.5% glucose solution by paper chromatography in the described manner. After 48 hours the paper was taken out, dried and sprayed first with 0.5% aqueous silver nitrate solution in acetone and then with 2-3% aqueous sodium hydroxide solution in alcohol. The spots were made permanent by washing the paper with dilute ammonia. The spots for the sample and B.D.H. inulin were exactly the same concerning wandering speed and intensity of the glucose and fructose parts, while the spot of the glucose sample was in line with the corresponding spots of the hydrolysates.

10. *Preparation of the Acetyl Derivative of C. Inulin by Haworth and Streight's Method.*—2 g. of the centaurea inulin was shaken with 20 ml. of freshly distilled pyridine in a round bottomed flask at 80-90°C. for about 45 minutes until the whole went in solution. After cooling, 4 ml. of freshly distilled acetic anhydide was added dropwise. The flask was covered with a calcium chloride tube and heated at 80-90°C. for about 6 hours on water bath. The clear solution was then poured into 200 ml. of water. The acetate was separated out as a white and somewhat gelatinous substance sticking to the walls. The aqueous solution was decanted off and the residue washed repeatedly with distilled water, dried and purified by dissolving in hot methyl alcohol, filtering and separation on cooling. Finally it was purified by dissolving in chloroform and precipitating with petroleum ether. This process was repeated ten times and then dried over phosphorous pentoxide in a vacuum desiccator. The rotation was $[\alpha]_D^{24} = -41°$ in 1% chloroform solution. Shrinking, swelling and foaming starts between 65-115°C.

at 115°C. It becomes viscous and melts completely at 140-150°C. to a colourless clear melt. The acetyl derivative was a white amorphous powder, easily soluble in chloroform and benzene, sparingly so in methyl alcohol and insoluble in petroleum ether and water.

In the same way the acetyl derivative of B.D.H. inulin was prepared. For data see Table 2.

Acknowledgement.—The authors are indebted to Dr. M. .M. Qurashi for carrying out the infrared spectrography. The microanalysis has been done by A. Bernhardt, Max Planck Institut Fuer Kohlenforschung, Muelheim, Ruhr, Germany.

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AN EXAMINATION OF AMOORA ROHITUKA

Part I.—Constituents of the Seed

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Constituents of the seed of the plant *Amoora Rohituka* have been investigated. An essential oil and a sterol have been obtained. The fixed oil after removal of the essential oil consisted of a mixture of saturated and unsaturated acids.

Introduction

The plant has been mentioned in Watt's Dictionary of Economic Products of India[1] as well as in Bengal Plants[2] and Flora of British India.[3] It appears to be synonymous with Amoora Tikta-raj and Andersonia Rohituka, locally known as Rohituka or Royna. It grows wild in various parts of East Pakistan, and is a medium sized ever green tree with a large crown of branches, compound leaves are imparipinnate and leaflets oblique. Flowers are subsessile, spicate; male spikes are branched, while the females are simple; calyx 5-partite. The fruits are about 1.5″ across, subglobose, coriaceous, loculicidally 3-valved capsules. Seeds are with fleshy bright arillus. The outer seed coat is black and glossy. Only some rudimentary work appears to have been done on the seeds and seed contents.[4,5,6,7] But these appear to be rather incomplete. There is no mention anywhere about the essential oil that has now been detected in it.

A sample quantity of seeds of this plant was obtained through the courtesy of the Conservator of Forests, Eastern Circle in East Pakistan and our preliminary work has been done with that sample.

The oil expressed from the seeds has been in use for a long time in East Bengal for burning purposes. It has also been sometimes used medicinally, as a massage oil in rheumatic pains. The seeds, however, do not seem to have any large scale industrial use at present. To explore the possibility of putting the oil to a more economic use, an investigation was undertaken.

The seeds on being extracted with petroleum ether yielded an oil, about forty eight per cent by weight. It contained an essential oil, which we have named Amoorin, that could be removed by distillation in steam. A sterol, has also been isolated after saponifying the oil[8] as a non-saponifiable constituent, which melts at 135.5°C. and its fuller examination is in progress. The fixed oil fraction that remained after the removal of the essential oil, has rather a high iodine value. It was expected, therefore, to contain highly unsaturated fatty acids and could have pronounced drying properties. Its examination revealed the fact that it consisted of about a quarter of its weight of saturated acids and three quarters of unsaturated acid mixture. The solid acid appears to be a mixture of stearic and palmitic acids, while the liquid fraction could be separated into oleic, linoleic and linolenic acids. The percentages of the acid constituents in the mixture have been shown in the following. The values are somewhat different from what have been recorded in the literature.[5]

From about 51 g. of the mixed acids obtained from the oil, the quantities of the constituent acids were approximately as follows:—

Palmitic Acid	4.64 g.	9.52%
Stearic Acid	7.4595 ,,	15.32%
Oleic Acid	8.1428 ,,	16.72%
Linoleic Acid	25.8681 ,,	53.11%
Linolenic Acid	2.5940 ,,	5.32%

Experimental

Constituents of the Seed.—100 g. of the seeds weighed 59.3 g. From these the kernels were separated consisting of 79.5% of the whole seed, while the seed coat consisted of 20.5% by weight. Results of analysis of the kernel are given below. These are very different from what Raoul Lecoq has recorded.[8]

Moisture	6.81%	Proteins	11.08%
Fats and oils	48.69%	Ash	2.74%
Carbohydrates	29.66%	Fibre	1.02%

Extraction of the Oil.—(a) The seed pulp was pressed in a local ghani, and about 36 per cent of the weight of the seed was obtained as oil. This possessed a very disagreeable odour. When the oil was mixed with petroleum ether a colourless precipitate was formed in small quantity, which settled on standing. This was washed with petroleum ether several times and has been kept for examination.

(*b*) Second method of isolation of the oil consisted in extraction of the powdered pulp with a solvent. For this 2.5 kg. of the kernel powdered and extracted with petroleum ether at the ordinary temperature by percolation, till practically the whole of the oil came out. The solution was treated with animal charcoal and the solvent was removed by distillation, when a residual oil 1.2 kg. was obtained. This was steam distilled, when the fixed oil was freed from an essential oil, which went over with steam. The fixed oil thus purified was freed from solvent, dried and analysed. It has sp. gr. 0.9187 at 25°C.; $[n]_D^{25}$ 1.4786; saponification value 180.2; iodine value 122.1, and acid value 13.1. The residual pulp is under examination now and results will be communicated in due course.

Essential oil Amoorin.—One part of the crude oil (1.2 kg.), was distilled in a current of steam for 4 hours, when an essential oil (5.1 g.) with a rather characteristic odour came along. This was isolated in the usual way. It was distilled under reduced pressure, when it boiled at 95°-107°/8 mm. On redistillation, most of it came over at 105°C./8 mm. It is a light yellow coloured mobile liquid with a peculiar odour. It has $[d]_4^{31}$, 0.9037 and $[n]_D^{31.5}$, 1.4931. It analysed for $C_{26}H_{42}O$. C, 84.42% and H, 11.32% was found while $C_{26}H_{42}O$ required C, 84.32% and H, 11.35%. Its molecular weight (by Rast method) found 377.2, required 370.6. This oil has been named "amoorin" and work on its constitution is now in progress.

Composition of the Fixed Oil of Royna.—75 g. of the oil was saponified with alcoholic potash; the dry potassium soap was extracted with ether to remove unsaponifiable matter, and then the soap was decomposed with hydrochloric acid. 69.5 g. of mixed fatty acids was thus obtained. Saturated and unsaturated acids in the fatty acid portion were estimated by Twitchell's lead-alcohol method.[9] Out of this 60 g. was dissolved in 300 cc. of 95% alcohol, and heated to boiling. To this, was added a boiling solution containing 42 g. of lead acetate in 300 cc. of 95% alcohol. The alcohol in both cases contained 1.5% of its weight of glacial acetic acid. The mixture was kept at room temperature overnight. The precipitate was then filtered and washed with cold 95% alcohol until the washings were free from lead. The precipitated lead salts were then dissolved in the same solvent as above, and the solution was cooled overnight. The undissolved precipitate was removed, washed with the same solvent and decomposed with dilute hydrochloric acid. The ethereal solution containing the solid fatty acids was then washed with distilled water, until the washings were no longer acid to methyl orange. The ether was then distilled off, the residue dried and weighed. Lead salt of the liquid fatty acid was recovered from alcoholic solution and treated in the same way. The mixed acid weighed 58.6 g. of which the saturated acid consisted of 14.78 g. or 25.2% and the unsaturated acid amounted to 43.92 g. or 74.8%.

The iodine value of the saturated acids was found to be 4, its molecular weight was 274.99, and it melted at 54.5° to 55.5°C. After several crystallisations from alcohol, the melting point of the saturated fatty acids gradually rose to 64.5°-65.5° when it had m.w. 281.45.

Examination of Unsaturated Acids.—The unsaturated acid was characterised through its bromine additive products as carried out by Eibner and Muggenthaler[10] recommended by Jamieson and Baughman.[11] For this purpose 2 g. of the acid was dissolved in 50 cc. of dry ether cooled in ice to 0°C.; bromine was then slowly added to it, and the mixture further cooled and allowed to stand for two hours at about −10°C. At this stage, no precipitate, insoluble in ether, was obtained. This indicated that no appreciable quantity of linolenic acid was present in the mixed unsaturated acids. This would be converted into its hexabromoderivative, which is almost insoluble in ether. The excess of bromine was then removed from the ethereal solution by washing it with an aqueous solution of sodium thiosulphate. It was dried over anhydrous sodium sulphate and the solvent was removed. The residue was then taken up with 100 cc. of petroleum ether (b.p. 60-80°C.) and kept in a cooler overnight. A precipitate of linolenic tetrabromide was obtained which on recrystallisation from xylene melted sharply at 114°C., which is the melting point of tetrabromostearic acid.

Separation of Fatty acids by the "Urea Adduct Method"[12] used by Mehta and Dabhade[13]:—50.95 g. of mixed fatty acid was added to a solution of 100 g. of powdered urea in 450 cc. of rectified spirit. The mixture was heated and kept overnight at room temperature. The adduct obtained was filtered. The filtrate was further concentrated

TABLE I.—FRACTIONATION OF MIXED FATTY ACIDS OF ROYNA OIL WITH UREA–STARTING MATERIAL: 50.95 g. MIXED FATTY ACID, 100 g. UREA, 450 cc. ALCOHOL.

Fractions	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Weight of fatty acid in each fraction	6.8745	5.0442	3.7411	3.3055	5.1560	4.5970	3.8821	16.1040
I. V.	6.75	14.29	53.86	104.70	154.61	174.70	184.92	195.00
M. w.	170.01	278.6	283.1	283.				
Saturated C_{16} (in g.)	3.53	1.04	0.07					
Saturated C_{18} (in g.)	2.8282	3.2022	1.4291					
Oleic	0.5163	0.8020	2.2420	2.7678	1.4952	0.3195		
Linoleic	—	—	—	0.5377	3.6602	4.2725	3.7192	13.6729
Linolenic	—	—	—	—	—	—	0.1629	2.4311

Result:—Palmitic Acid – 4.64 g. or 9.52%. Linoleic Acid – 25.8681 „ „ 53.11%.
Stearic Acid – 7.4595 „ „ 15.32%. Linolenic Acid – 2.5940 „ „ 5.32%.
Oleic Acid – 8.2428 „ „ 16.72%. Total – 48.8044

under reduced pressure and was allowed to crystallise at room temperature. The precipitate obtained was treated similarly. This fractionation technique was repeated a number of times. Each of the fractions obtained was treated with acidulated distilled water and extracted with ether and washed free of mineral acid. The ether extract was dried over anhydrous sodium sulphate and the solvent was removed in the usual way. Each of these fractions was then examined for iodine and neutralization values.

The fatty acid composition of each fraction was calculated from the iodine and neutralization values by assuming the fractions to be binary mixtures. The mean molecular weight of the saturated acid in each fraction could be calculated from the neutralization values. The results are enumerated in Table I.

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SHORT COMMUNICATIONS

A NEW METHOD FOR REFINING OF SHARK LIVER OIL ON COMMERCIAL SCALE AND RECOVERY OF VITAMIN 'A' BY PARTIAL SAPONIFICATION

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Introduction

Despite synthetic preparation, fish liver oil will continue to be a potential source of vitamin A in the world market. The shark liver oil industry in Pakistan has a great future. The methods for extraction and refining of shark liver oil as practised in other countries do not however seem economical in this country. Sharks from tropical waters yield liver oil comparatively of higher melting point. Only a small fraction of oil can be obtained in liquid state when wintered at 20-22°C. The major portion of crude oil remains solid, and unless vitamin A could be recovered from solid fraction easily, the liver oil industry will not be a sound proposition.

The method described in the present communication is inexpensive and easy to operate without much installation. It works on the principle of extraction of vitamins A and D from partially saponified crude oil through the medium of cottonseed or any liquid vegetable oil. By extracting three times in succession, a transference of vitamin A to the extent of 95% has been achieved. For partial saponification, caustic potash or caustic soda may be used, although the former works better. A patent has been applied for in Pakistan under Patent Application No. 1082/59, dated 3.12.1959.

Experimental

In a 15-literdrum, provided with two taps, were placed 5 lbs. of crude shark liver oil (4,000 I.U./g.). 145 g. of caustic soda dissolved in 150 ml. of water was introduced and stirred thoroughly. The mixture was allowed to stand for 48 hours. Five lbs. of cottonseed oil heated to 60°C. was added and the whole made into a uniform mass by stirring. This was allowed to stand for 4 hours, and 2 liters of hot saturated brine was then added to precipitate the soap into a granular mass. After standing for 24 hours, 5 lbs. of the supernatant liquid oil was removed through the upper tap and was collected in a second drum with two taps as before. The oil, washed first with hot brine (90°C.) and then with hot water, was separated by the upper tap and centrifuged in an oil centrifuge. This was termed as first extract. The potency of the oil was determined chemically using colorimetric comparison on the Lovibond tintometer, and found to be 2,500 I.U./g. Transference of vitamin was 62%. To the residual mass a further quantity of 5 lbs. of fresh cottonseed oil heated to 60°C. was added. The mass was stirred thoroughly and allowed to stand overnight. Five lbs. of oil was removed, washed and centrifuged as before. The potency of this second extract was determined and found to be 1,000 I.U./g. Transference of vitamin in this case was 25%.

The residual mass was treated for the third time with another lot of fresh cottonseed oil. The oil recovered was 5 lbs. with a potency of 300 I.U./g. Transference of vitamin in this case was 8%. The total transference of vitamin by these three partial treatments was 95%. The solidifying point of the crude oil was 30-35°C. and that of the product was 12°C.

By blending the oil impregnated with vitamin A obtained by the above treatments a standard product of average potency was produced. The third extract having a low vitamin potency was used for transference of vitamin in subsequent operations. To prevent any deposition of "solid oil" in cold weather, the oil was wintered at 16°C. The wintering period was 5 days at a stretch.

In the above experiment saponification had also been effected by caustic potash. The soap in this case separated from the unsaponified oil and was found as a separate layer at the bottom. No brine treatment was necessary. Separation of oil-solvent used for transference of vitamin proceeded with greater ease. Percentage of transference of vitamin A in the first extract was found to be slightly higher than that with caustic soda. The residue was utilised for the production of cheap quality washing soap as by-product.

The procedure was further utilised to produce

higher potency oil from lower one. Vitamin-bearing oil recovered as the first extract was used for transference of vitamin in the second operation. By extending the process to next operation a higher potency oil was produced.

Summary

This is a process for refining and production of shark liver oil by partial saponification either with caustic soda or caustic potash. In this process cottonseed oil was used as an oil solvent for transference of vitamin from crude shark liver oil.

Higher vitamin A potency oil was also produced from lower potency crude shark liver oil by using this process.

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EUBLEMMA AMABILIS

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(Received December 1, 1959)

In a systematic cultivation of lac, the first point to remember is that it should be intensive. It means that there should be a forest-plan for the particular lac-plantation or lakhera, and naturally this cannot apply generally to each and every locality even when the insect and its host tree remain the same. The forest-plan will be an integration of numerous factors, some promoting the multiplication of the insect and others detrimental to its life, being a sort of balance sheet to indicate how favourable the enterprize is likely to be in the end.

Coming to the factors destructive to lac, a serious one is the attack from the predacious caterpillars of *Eublemma amabilis* moth. The destruction due to this enemy alone has to be quantitatively worked out and, before we can proceed with it, its population with each crop has to be calculated. No such attempt has been undertaken because of the difficulty of counting the caterpillars in a given specimen of fresh stick-lac where the lac insects are still alive. Normally the caterpillars of *E. amabilis* eat beneath the cover of the lac crust. This is apparently due to the moth larvae shunning light. When an isolated piece of fresh stick-lac is placed in a wooden box, dark and yet sufficiently airy, the caterpillars, instead of remaining within the lac, form perpendicular columns of tunnels projecting outside the twig.

In Mysore, the insect, *Lakshadia, mysorensis*, growing there on *Shorea talura*, suffers much from the attack of *E. amabilis*. As such, any given sample of fresh lac would contain sufficient predacious caterpillars of this moth. Figure 1 shows such a specimen with rod-like projections arising perpendicularly to the length of the twig. These

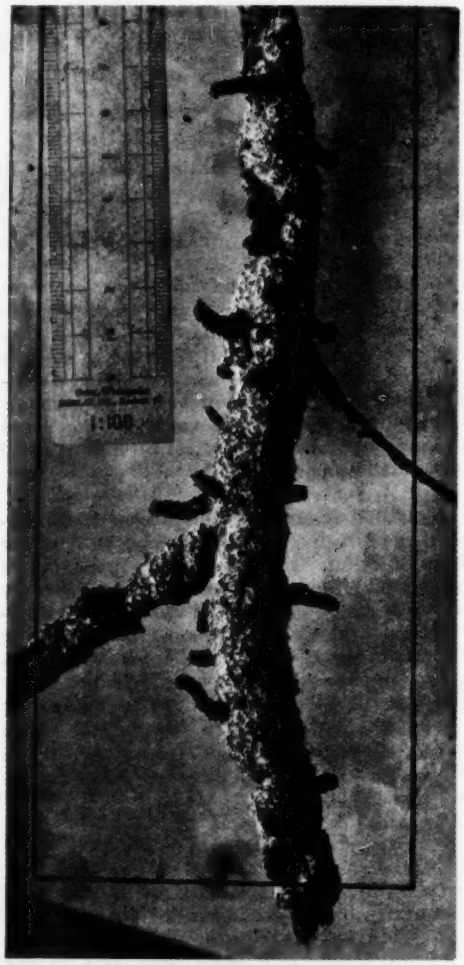
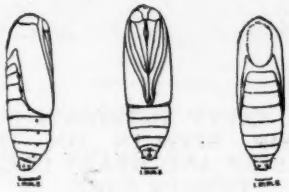


Fig. 1.—Fresh stick-lac of *Lakshadai mysorensis*, on *Shorea talura*, Bangalore. Sample of stick collected at the end of the rainy season. The worm-like projections are hollow tubes constructed by the caterpillar of *Eublemma amabilis*. They are made of resinous excreta of the caterpillar incorporated in a web-tube secreted by the insect. The scale shows centimetres.

Fig. 2.—Caterpillar of *E. amabilis*, not full grown yet, with its anal end half ejecting a disc shaped excreta seen sideways.



(a) (b) (c)

Fig. 4.—The pupa of *E. amabilis* :— (a), seen sideways ; (b), from its ventral side; (c), from above.

projections are formed by the *E. amabilis* caterpillars, which secrete silken thread and bind their excreta together to construct hollow tube-like tunnels within which they move. Normally these tubes are hidden between the lac crust and the surface of the twig. When otherwise exposed, as in Fig. 1, the population of living caterpillars is at once ascertained, which is a very helpful device in quantitatively estimating the injury due to this enemy alone. I have not found any other enemy-caterpillar behaving likewise, certainly not that of *Holcocera pulverea*, which is also common in several localities. This caterpillar also forms a silken cacoon but purely of silk, and a very loose

Fig. 3.—*E. amabilis* caterpillar, full grown, to the right. To the left a ball shaped object, the last moult skin of the caterpillar, with an oval dark object, the excreta.

(a) (b) (c)

Fig. 5.—Pupa of *E. amabilis*, its posterior end enlarged. (a), seen sideways; (b), from beneath; (c), from above.

one. In the architecture of its cacoon, its excreta is not incorporated; this is of a granular nature hardly capable of offering any strength to a tube-like construction.

Figure 2 shows the caterpillar of *E. amabilis* not yet full grown. The anal end at right shows a disc half projecting out of the anus. It is the excreta of the caterpillar seen in profile. In

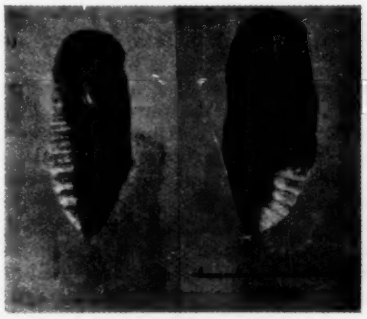


Fig. 6.—Pupae of *E. scitula*, that of male to the left, that of female to the right.

(a) (b)

Fig. 7.—Moths of *E. amabilis*, almost natural size. (a), the normal moth, deeply coloured; (b), from a caterpillar feeding on lac attacked by *Erencyrtus dewitzii*, the moth being yellow, instead of pink.

Fig. 3, at right is shown a full grown caterpillar, and at left a ball shaped object represents the moulted skin of the caterpillar before it became a pupa, with an oval dark spot, a single disc of excreta, which was seen sideways half ejected in Fig. 2. The malpigian tubes of the lac insect, contain the ether soluble portion of lac resin which is soft or semi-fluid but easily dries to a brittle surface when smeared on a microscopic slide. When the caterpillar of *E. amabilis* eats the lac insect it also feeds upon the contents of the malpigian tube which is the "soft resin" and it is this which is excreted as a hard disc. It may be noted that lac when burnt gives a pleasant butter-like smell, and on dry distillation of lac butyric acid has also been recovered. There is no doubt that the ether soluble constituent of lac is first to be found in the malpigian tube. When finally excreted it comes as flat discs, which are hard and severe, so to say, as bricks, which joined together by a silken web secreted by the caterpillar, goes to form a tube or tunnel, within which it moves while it is feeding more and more on the lac insects.

The same specimen, Fig. 1, for example, can be further dissected later on to see how many pupae of the moth were there, to supplement the finding based on caterpillars alone. Figure 4 gives a pupa of *E. amabilis* as seen in three poses: Fig. 4a, shows it sideways; Fig. 4b, ventrally; and Fig. 4c, dorsally. The anal end of the pupa is shown further enlarged in Fig. 5. Figure 5a gives the sideview; Fig. 5b, its ventral and Fig. 5c, its dorsal aspect.

From 1954 to 1959, I have reared parasites from both the annual crops and it has been my impression that *Eublemma amabilis* is least common in Sind. However, from no other lac could I breed *E. scitula* more than from Sind lac (pupae shown in Fig. 6. for comparison); it is fortunately rare even here. The caterpillars of *E. scitula* hardly feed underneath the lac encrustation. They usually devour young lac insects and are, so to say, surface feeders. The injury due to this enemy alone is difficult to ascertain and is a problem to be solved in the future.

The caterpillars feeding on lac insects normally containing the red lac dye ultimately give rise to moths coloured pink. Imms and Chatterjee[1] have given a colour picture of the moth, *E. amabilis*. There could be other factors besides the lac dye which may contribute to the depth of colour shown by the moth. Specimens of moth from Mysore were deeper in shade than that illustrated by Imms and Chatterjee. Figure 7a gives a natural size picture of this moth. In an experimental inoculation of the species, *Lakshadia communis*, on *Guazuma tomentosa*, at Bangalore, the third larval stage was severely attacked by the chalcid *Erencyrtus dewitzii*. A caterpillar of *E. amabilis* feeding upon such food got little lac dye and the moth reared from it was pale yellow with just a tinge of pink at the edge of the markings. This abnormal pale moth looked quite different in colour and is shown as Fig. 7b; it contrasts with the normal red specimen, Fig. 7a. It confirms the role of the red dye in the food in producing the deep colouration of the normal moth Fig. 7a.

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ESSENTIAL FATTY NUTRIENTS IN DIETS AND THEIR EFFECTS ON HEALTH, AND ON LIVER AND HEART DEGENERATION IN MICE

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Diet is one of the important factors in coronary heart disease[1] and liver degeneration.[2] Through short period experiment the fats in diets have been found to cause many ailments.[3-4] Peacock[5] has elucidated the effects of dietary fatty materials on the animal systems. In the present investigation, with long-term animal experiments the stable fat of the diet containing essential fatty acids (EFA), tocopherols, and mixture of ascorbic and citric acids, has been shown to play crucial roles on these phases of ailments.

The short-period experiments [3-5] do not reveal the cumulative effects of fats and the allied substances. The present series of experiments were, therefore, extended over the whole life span of the healthy and genetically pure male C_3H mice (40 per series). All precautions were taken to eliminate causes of diarrhoea, external infections, sudden shock and panic. The mice were housed in cages having twenty in each. The hydrogenated cottonseed oil blended with 8% original cottonseed oil, was obtained from a commercial firm. One batch of cottonseed oil was heated at 180°C. with metals (cast iron, aluminium, stainless steel, monel and copper) for about four hours for establishing the aggregate effects of cooking vessels. The oil was oxidized to one per cent peroxide contents by bubbling oxygen in presence of visible

light. In order to maintain homogeneity in oils used, required quantities of cottonseed oil for all experiments were purchased at one time and stored below o°C. The weekly diet was prepared with a fixed composition (experimental fat, 17.5; crude casein, 21.7; sucrose, 10.1; glucose, 14.1; starch, 17.1; brewer's yeast, 8.2; alfalfa meal, 4.1; salt mixture, 4.1; cod liver oil, 2.0 and wheat germ oil, 1.1%), stored below o°C., fed *ad libitum* and weekly growth records were maintained. Within a month by gradual increase the mice were made accustomed to 1.0% peroxidized fat. However, these mice started dying from the third month and within six months all died due to heart failure. The hydrogenated cottonseed oil fed mice exhibited sound health, those fed on plain cottonseed oil medium health, and those fed on heated cottonseed oil, stunted growth. The mice were killed under ether and opened for observations.

Results are summarised in Table 1. Evidently, the stability of tocopherols in dietary and metabolic fats, plays the main role in the health of mice. The peroxidized fats destroyed tocopherols, resulting in faulty fat metabolism[3] and heart failure. The heated fats with unstable tocopherols[6] caused damage to both heart and liver. Cottonseed oil was not as stable as the hydrogenated one and the hydrogenated oil containing mixture of ascorbic and citric acids. The latter samples with stable fats and tocopherols did not affect heart, but the liver cell proliferation was very severe in one and not so much in the other (Table 1). It seems that tocopherols and fats (with EFA) stabilize each other *in vivo* as *in vitro*[7] and maintain uniform growth in all organs of mice, except liver having uncontrolled growth of cells. Probably, some inhibition of such excess proliferation, by ascorbic and citric acids, may be related to enhancement of tocopherol efficiency as biological antioxidant with cellular control mechanism preserved.

TABLE 1.—TOCOPHEROLS IN DIETARY FATS AND HEALTH OF C_3H MICE.

Experimental fats used in the diets	Tocopherol contents in fats γ */g.	Heart failure		Liver cell prolifer-ation	
		Incidence %	Duration (months)	% animals having initiation	Duration
HCO*	824.0	0.0	12½	92.0	12½
HCO+Ascorbic & citric acids**	938.0	0.0	12½	92.0	12½
CO	1120.0	15.0	12½	45.0	12½
Heated CO	135.0	37.0	12½	29.0	12½
CO with 1.0% peroxides by weight of CO	0.0	100.0	6.0	0.0	6.0

* HCO ; Hydrogenated Cottonseed oil; CO : Cottonseed oil; microgram: γ.

** 60 mg. Ascorbic and 150 mg. citric acid per 100g. of diet.

In another series of preliminary experiments, excess tocopherols, eight times in amounts, in comparison to those present in hydrogenated cottonseed oil (HCO) isolated from cottonseed oil (CO) and added to hydrogenated cottonseed oil, caused stunted growth in mice. It seems that excess tocopherols initiate autoxidation[7] *in vivo* and deteriorate EFA[1,7,9] producing faulty fat metabolism.[3,10]

These findings indicate interesting facets of aging and ailing processes, although extreme caution must be employed in translating data from one species to another. Tocopherols as biological antioxidants *in vivo* with excess becoming prooxidants, as *in vitro*[7], may be influenced[1,3] by choline, lecithin, phosphatides, amines, inositol and pyridoxins, amidst[3] glutathione, ascorbic, citric and phosphoric acids in correcting the faulty fat metabolism[3] in which EFA, vitamins A and pantothenic acid, may have significant roles.[1,8] Such influence on tocopherols is governed by the antioxidant and synergistic actions of these substances[1,3,10,11] affecting the autoxidation behaviour of EFA and vitamin A.[1,11] Pantothenic acid helps metabolism of fats[12] in conjunction with EFA.[3,13]

The proportionate amounts of foods[8,14,15] (balanced diet in particular) are important in the maintenance of health free of ailments. Consequently, dietary bulk and ratio of carbohydrate, protein, and fat with considerations for ingestion[8] of the above and other nutrients, physiologically active agents and nutrient-antagonists (say, in the form of preservatives, flavours, colours, additives and luxurious uses of plastic and polymer[8] utensils in foods and food preparations) may reflect the ailing and aging mechanisms after due reservations for heredity, sex life's stress and strain. These points are discussed in the foregoing sections, in order to present a coherent picture of the field and its essentials.

Now considering the present day luxurious and lazy approach to ready-made foods with many contaminants,[8] a prediction should be made for stimulating people's cautiousness and also for further investigation. Probably, by 1970, man may present many medical problems, because man's system takes more than 20 years to exhibit any abnormal effects.[8,16]

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NOTES & NEWS

Pakistan's First Steel Mill

The first steel mill of Pakistan will be located in Karachi. The decision to set up the mill in Karachi on a comparatively small scale was taken up by the Economic Committee of the Presidential Cabinet.

The installation of this small scale steel mill in Karachi would not, in any way, mean the shelving of plans for a full-fledged mill at Multan.

The mill will be set up soon and is expected to go into production sometime during the current year. It is expected to have a production capacity of 70,000 to 80,000 tons a year. The estimated cost of the mill was not worked out finally, but it may well be over Rs. 1,00,00,000. To start with, the local scrap and imported pig iron would be used in the mill. Local raw iron may also be used provided it is found to be of good quality and could be converted into pig iron economically. Installation of the mill will form part of the Second Five Year Plan.

Recommendations of the Scientific Commission of Pakistan

The Scientific Commission, which concluded its deliberations in Karachi in December 1960, has recommended the creation of a high-powered Science Foundation for promoting and coordinating fundamental research at all levels. The Commission also recommended the desirability of keeping the Foundation as an autonomous body under the direct charge of the executive head of the country so that it may fnuction independent of any particular Ministry. The Foundation may be created under a statutory charter.

The Science Foundation may have the following five constituent units, each dealing with subjects of most vital importance to the country:—

1. Food and Agricultural Research Council.
2. Medical Research Council.
3. Industrial Research Council.
4. Atomic Energy Research Council.
5. Irrigation and Works Research Council.

Each of these constituent units may be autonomous except to the extent that the financial allocation for each unit shall be determined by the Science Foundation. Each unit will be responsible for establishing research institutes and laboratories in both wings of the country.

The Scientific Commission reached an important conclusion that all scientific research should be the direct responsiblity of the Central Government.

With a view to making the scientific and technical careers in Pakistan more charming, the scientific Commission has also emphasised the desirability of the establishment of a service cadre of scientists on the lines of the civil service. This cadre is likely to include technical workers engaged in research problems under the overall discipline of science.

Establishment of a Lac Research Institute

In accordance with the decision of the Governing Body of the Pakistan Council of Scientific and Industrial Research, a Research Institute for Lac, Wax and Fibre will be set up in Rajshahi, East Pakistan. The Rajshahi area is considered to be most suitable for cultivation of lac, which is at present imported from abroad.

Forthcoming International Conferences

13th International Symposium on Crop Protection; May 7, 1961; Ghent, Belgium.

International Road Tar Conference; May 29-31, 1961; Harrogate, Yorks, U.K.

5th Congress of the European Molecular Spectroscopy Group; May 29 to June 3, 1961; Amsterdam, Netherlands.

9th International Colloquium on Spectroscopy, (GAMS); June 5-10, 1961; Lyon, France.

General Assembly of the International Organization for Standardization, (Members only); June 8-18, 1961; Finland.

International Conference on Methods of Direct Separation and Chromatography, (GAMS); June 13-14, 1961; Paris, France.

3rd International Symposium on Gas Chromatography; June 13-16, 1961; East Lansing, Michigan, U.S.A.

International Plastics Exhibition and Convention; June 21 to July 1, 1961; London.

8th Congress of the International Union of Leather Chemists Societies; June 25-30, 1961; Washington, D.C., U.S.A.

Annual Meeting of the American Medical Association; June 26-30, 1961; New York, U.S.A.

8th International Gas Conference; June 28-30, 1961; Stockholm, Sweden.

International Symposium on Inorganic Polymers; July 18-21, 1961; University of Nottingham, Nottingham, U.K.

International Symposium on Macromolecular Chemistry; July 27 to August 1, 1961; Montreal, Canada.

21st Conference of the International Union of Pure and Applied Chemistry; August 2-5, 1961; Montreal, Canada.

5th Internationl Congress of Biochemistry; August 6, 1961; Moscow, U.S.S.R.

14th Congress of the International Association of Applied Psychology; August 9, 1961; Copenhagen, Denmark.

10th Pacific Science Congress; August 21 to September 2, 1961; Honolulu, Hawai , U.S.A.

6th International Conference on Co-ordination Chemistry; August 27 to September 1, 1961; Detroit, Michigan, U.S.A.

Symposium on "Machine Translation of Languages and Applied Language Analysis"; September 5-8, 1961; National Physical Laboratory Teddington, U.K.

4th International Seaweed Symposium; September 18-25, 1961; Rome, Italy.

31st Annual International Meeting of the Society of Exploration Geophysicists; November 5-9, 1961; Denver, Colorado, U.S.A.

4th Conference on Applied Meteorology; November 8-11, 1961; Atlanta, Ga., U.S.A.

14th International Symposium on Crop Protection; May 2, 1962; Ghent, Belgium.

2nd International Rayon and Synthetic Fibres Committee Congress; May 15-18, 1962; London, U.K.

4th Rubber Technology Conference; May 22-25, 1962; London, U.K.

4th International Congress on Coal Preparation; May 28 to June 1, 1962; Harrogate, Yorks, U.K.

7th International Conference on Co-ordination Chemistry; June 25-29, 1962; Stockholm, Sweden.

6th International Congress on Glass; July 9-14, 1962; Washington, D.C., U.S.A.

12th International Congress of Mathematicians; August 15-22, 1962; Stockholm, Sweden.

International Congress of Microbiology; August 19-25; 1962; Montreal, Canada.

10th International Congress of the History of Science; August 26 to September 2, 1962; Ithaca, New York, U.S.A.

5th International Congress of Electron Microscopy; August 29 to September 5, 1962; Philadelphia, Pa., U.S.A.

6th (Plenary) World Power Conference; October 20-26, 1962; Melbourne, Australia.

3rd World Metallurgical Congress; November 5-9, 1962; Chicago, Ill., U.S.A.

International Society of Soil Science — Commission 4 and Commission 5 — Joint Meeting; November 13-21, 1962; Palmerston North, New Zealand.

BOOK NOTICES

Microanalysis by the Ring Oven Technique. Herbert Weisz. 120 pp. Pergamon Press, London, New York, Paris, Los Angeles, 1960. Price 30s. ($4.50).

Microanalysis by the Ring Oven Technique deals with a technique originally developed as a qualitative separation technique for extremely minute samples, but which has found wide application in different branches of analytical chemistry. Within the past few years, it has been extended to semiquantitive analysis, to the analysis of radioactive substances, to electrographic analysis, and the like.

In qualitative or quantitative analysis, separation of the substances contained in the sample into one or more groups is one of the most important steps. The separation steps must be selected in such a way that the substances which are collected together in one group do not interfere with the subsequent identification or determination of each substance in the groups.

The successful application of the ring oven technique under such conditions is fully described in this volume. Full details of apparatus, methods and their application and possibilities for future development are given, and there is a useful bibliography.

The Collected Papers of Paul Ehrlich. Vol. Iii Chemotherapy. Edited by F. Himmelweit 625 pp. approx. Pergamon Press, London New York, Paris, Los Angeles, 1960. Price £ .8 ($ .25).

The Collected Papers of Paul Ehrlich, Volume III, published under the editorial direction of Sir Henry Dale, and with the assistance of the Wellcome Trust, contains the published records of Ehrlich's researches on chemotherapy. Chemotherapy—the search for artificial synthetic remdies acting directly, by virtue of their specific chemical affinities, upon the organisms responsible for the diseases of infection—was regarded by Ehrlich himself as the ultimate aim and, eventually, as the crowning achievement, towards which the whole course of his researches had been primarily directed.

Volume I covered his work on Histology, Biochemistry and Pathology and Volume II Immunology and Cancer Research.

Ehrlich's discoveries and theories are to a large extent responsible for the astonishing advance in medical knowledge and its applications during the past half-century, and this series is of absorbing interest to all connected with medicine.

The Eltectrolytic and Chemical Polishing of Metals (in Research and Industry). W. J. McG. Tegart. 140 pp. Pergamon Press, London, New, York, Paris, Los Angeles, 1959. Price 40s. ($ 6.50).

The Electrolytic and Chemical Polishing of Metals in reserach and industry gives a comprehensive survey of those processes used both as metallurgical techniques and as industrial finishing operations.

Apparatus, techniques, precautions required and limitations of the process are all discussed in relation to such topics as electropolishing in molten electrolytes, and electrolytic and chemical polishing of some of the rarer metals and their alloys.

This new edition has been extensively revised and amongst the more important revisions are a fulier discussion of the nature of mechanically prepared surfaces and of commercial electropolishing units, many more illustrations, and an up-to-date reference list.

Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry. L.M. Jackman. 134 pp. Pergamon Press, London, New York, Paris, Los Angeles, 1959. Price 35s. ($ 5.50).

This book has been written for organic chemists who wish to employ nuclear magnetic resonance but who do not wish to make a detailed study of the fundamental principles involved. Accordingly the theory of nuclear resonance has been developed non-mathematically and only to an extent sufficient for the needs of the organic chemist. Much of the book is devoted to the presentation of correlated data and empirical theories which constitute the basis of the applicaion of nuclear magnetic resonance spectroscopy to organic chemistry.

This is the first book in which really precise correlations between chemical shifts and molecular

structure have been presented. It will be invaluable as a guide to the way in which the organic chemist will use nuclear magnetic resonance now and in the future.

Partial Table of Contents: Introduction and historical review. The theory of nuclear magnetic resonance spectroscopy. The experimental method. The correlation of the chemical shift with molecular structure. The interpretation of the spectra of complex organic molecules. Diamagnetic anisotropy and stereochemistry.

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